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Award Number: DAMD17-98-1-8621

TITLE: Neurotoxins and Neurodegenerative Disorders in Japanese-American Men Living in Hawaii

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REPORT DATE: October 2002

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

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20030731 279

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquart
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Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington,

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 2002	Final (21 September 1998 - 20 September 2002)

4. TITLE AND SUBTITLE

Neurotoxins and Neurodegenerative Disorders in Japanese-American Men Living in Hawaii

5. FUNDING NUNUMBER

DAMD17-98-1-8621

6. AUTHOR(S)

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

This is a program of epidemiologic and neuropathologic research aimed toward determining neurotoxic and preventive/ameliorative risk factors for Parkinson's disease (PD), parkinsonism, and other neurodegenerative conditions using existing data and autopsied brain tissue from the Honolulu Heart Program / Honolulu-Asia Aging Study. Major findings from the four years of this include: a) a significant association of coffee and caffeine consumption with reduced risk of PD, b) an association of years worked on a plantation and occupation in sugar mill processing with increased risk of PD, c) an association of constipation with development of future PD, d) a significant association of increased triceps skinfold thickness measured in mid-life with future development of Parkinson's disease, e) an association of slow reaction time with presence of incidental Lewy bodies found in the substantia nigra or locus ceruleus at autopsy, f) an association of elevated GFAP in frozen brain tissue with lower cognitive function, and g) an association of prolonged QT interval with future risk of PD project. A genetics component found no association of CYP2D6 gene, the CYP1A2 gene, the dopamine transporter gene, the parkin gene, and the adenosine receptor gene with PD. Organochlorine levels from two areas of brain from each of 15 research subjects with the highest exposure to pesticides are complete and indicate measureable amounts of these compounds in most brains. Request has been made for supplement funds to perform organochlorine levels on all autopsied brains.

14. SUBJECT TERMS

Neurotoxin, Parkinson's disease, neurodegenerative disorders

15. NUMBER OF PAGES

232

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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INTRODUCTION

This is the final report for this project entitled "Neurotoxins and neurodegenerative disorders in Japanese-American men living in Hawaii". The goal of this epidemiologic and neuropathologic research program is to determine neurotoxic and preventive/ameliorative risk factors for Parkinson's disease, parkinsonism, and other neurodegenerative conditions. The research is an extension of the Honolulu Heart Program/Honolulu-Asia Aging Study (HHP/HAAS), a longitudinal study of heart disease, stroke, and dementia in a cohort of Japanese-American men born 1900-1919 who were living in Hawaii when the study began in 1965. Additionally, this project builds on a National Institute of Neurological Disease and Stroke funded study during which all cases of Parkinson's disease were identified in the HHP/HAAS cohort up to 1994 and smoking and dietary antecedents of Parkinson's disease were examined.

Components 1 and 2 of this research are identification of risk factors for Parkinson's disease (1) and parkinsonism (2) using existing data from the longitudinal HHP/HAAS. The work seeks to confirm previous reports [1-6] of an association of pesticide exposure with Parkinson's disease by examining the role of exposure to neurotoxins through occupational exposures such as sugar or pineapple plantation work (pesticides, herbicides) and self reported exposures to pesticides, metals, and other chemicals. Cases of Parkinson's disease in the HAAS cohort were initially identified 1991-1994. Since then new cases have been identified through self report and subsequent record review by a neurologist (the P.I.). Additionally, work is ongoing (through additional funding) to re-screen the HAAS cohort to identify new cases of PD and parkinsonism. Cases of parkinsonism are identified from a sample of the cohort who received the Unified Parkinson's Disease Rating Scale (UPDRS).

The neuropathological component, (#3) currently has access to over 480 brains from deceased HHP/HAAS participants. Lewy bodies in the brainstem pigmented nuclei have been identified and are being used as an endpoint in risk factor analyses. There are 43 brains that have Lewy bodies in either the substantia nigra or the locus ceruleus from participants that had no history of Parkinson's disease during life (incidental Lewy bodies). Additionally, new blocks of tissue have been cut from the periventricular white matter to assess the degree of demyelination and to determine the mechanisms leading to demyelination. Cerebral white matter change or leukoaraiosis found on CT or magnetic resonance brain imaging has been associated with aging, cognitive impairment, gait abnormalities and high blood pressure [7,8]. Damage to central nervous system myelin may be mediated by a variety of pathologic agents and processes such as ionizing radiation, toxins including organophosphates, infection, trauma, nutritional disorders, and ischemia [9]. Markers of brain injury such as glial fibrillary acidic protein and levels of organochlorine compounds are being evaluated using frozen brain tissue.

The 4th and final component of the research involves genetic determinants of Parkinson's disease. These are being investigated with collaborators at Stanford University in a case control study aimed at determining polymorphisms of the CYP2D6, dopamine transporter, CYP1A2, parkin, adenosine receptor, dopamine D2 receptor, paraoxonase 1, and VMAT genes that may be associated with Parkinson's disease. [10-17]

BODY: (numbers refer to items in the statement of work)

1, Evaluation of epidemiological risk factors for Parkinson's disease

A. Dietary factors and Parkinson's disease

1. Coffee and caffeine:

An abstract was presented at the American Academy of neurology in Toronto, Ontario (1999) titled: "Mid-life Coffee Consumption Is Inversely and Independently Associated with the Subsequent Diagnosis of Parkinson's Disease in Japanese-American Men" by Dr. Lon White. This work was later published in the Journal of the American Medical Association in 2000(Appendix A). Coffee drinking assessed at the baseline examination in 1965 and at the third examination in 1971 among the participants in the Honolulu Heart Program was found to be inversely related to the future development of Parkinson's Disease. A dose response pattern was found. Based on 30 years of follow-up since baseline examination, age-adjusted 10-year incidence of Parkinson's disease declined consistently with increased amounts of coffee intake from 9.4/1000 in men who drank no coffee to 1.7/1000 in men who drank ≥28 oz. per day ($p<0.001$). Similar relationships were observed with total caffeine intake and caffeine from non-coffee sources. Consumption of increasing amounts of coffee was also associated with lower risk of Parkinson's disease in men who were never, past, and current smokers at baseline. Other nutrients in coffee, including niacin, were unrelated to Parkinson's disease incidence. The relationship between caffeine and Parkinson's disease was unaltered by intake of milk and sugar and was independent of alcohol consumption.

2. Other dietary factors – foods

a. Fruit and fruit drinks

Increased fruit (relative risk = 1.55; 95% confidence interval = 0.83-2.89) and fruit drink (relative risk = 1.98; 95% confidence interval = 1.26-3.10) consumption increased PD risk after adjusting for age, smoking, coffee drinking , bowel movement frequency and history of working on a plantation. High dietary vitamin C intake was not associated with PD risk, after controlling for age, bowel frequency, smoking and coffee drinking. Likewise, use of vitamin C supplementation did not appear to be associated with PD risk. Please see manuscript by Dr. Andrew Grandinetti (appendix B).

b. Other foods

The 2000 annual report included analyses of 22 other dietary constituents possibly related to Parkinson's disease risk. There were 7916 participants (115 PD cases) who completed the 24 hour dietary recall during the 1965 examination. Below are the results of a series of models examining the risk ratio for dietary variables and PD each controlling for coffee consumption and cigarette smoking. The risk ratios shown represent the increment in risk associated with one unit served. There were no significant relationships between consumption of any of these foods with PD.

Dietary Variable – unit served	**Risk ratio**	**P value**
Whole milk – 1 oz	1.01	0.42
Ice cream – ½ cup	0.93	0.71
Cheese – 1 oz	0.85	0.59
Eggs – 1	1.0	0.98
Beef/lamb – 1 oz	1.0	0.97
Chicken/turkey – 1 oz	1.04	0.36
Pork – 1 oz	1.05	0.34
Bacon – 1 slice	0.8	0.18

Sausage – 1 oz	1.01	0.94
Cooked fish – 1 oz	1.0	0.93
Raw fish – 1 oz	1.04	0.68
Cooked rice – ½ cup	1.01	0.89
Noodles – ½ cup	0.86	0.22
Bread – 1 slice	0.97	0.57
Tofu – ¼ cup	1.05	0.61
Soy sauce – 1 tsp	1.01	0.72
Peanut butter – 1 tsp	0.94	0.33
Corn oil – 1 tsp	0.72	0.19
Butter – 1 tsp	0.93	0.38
Mayonnaise – 1 tsp	0.98	0.6
Sugar – 1 tsp	0.97	0.36

3. Other dietary factors – specific nutrients

Dietary data collected at the time of study enrollment (1965-1968) with 30 years of follow-up for the first appearance of clinical PD was used to calculate macro– and micronutrient intake. Based on the calculation of nutrient intake from the 24-hour recall data, total caloric intake, protein, niacin, riboflavin, beta-carotene, vitamins A, B, and C, dietary cholesterol, cobalamin, α-tocopherol, and pantothenic acid had no clear relation with clinical PD. Although the intake of vitamin E in the Honolulu-Asia Aging Study was modestly related to a reduced odds of PD, legumes (a food rich in vitamin E) were associated with a marked protective effect. Associations appeared for other dietary variables, but most consistently in subjects who were nonsmokers and nondrinkers of coffee. Please see attached manuscript (Appendix C). This work will be presented at the 11th Symposium for the Treatment of Parkinson's Disease to be held in Tokyo, Japan, October, 2002 and will be published in the Journal of Neurology.

Consumption of carbohydrates increased the risk of PD while the intake of polyunsaturated fats appeared protective. These relationships were strongest among non-coffee drinkers and non-smokers. The protective effects of coffee drinking and cigarette smoking are so strong that they mask the effects of other factors. Please see manuscript by Dr. Rob Abbott (Appendix C) for details. For carbohydrates PD incidence rose significantly with increasing intake for both non coffee drinkers and never smokers ($p<0.05$). Differences in the risk of PD, however, were modest up to the 4th and 5th quintiles of carbohydrate intake. In contrast, the intake of polyunsaturated fats appeared protective against PD, particularly in men who never smoked cigarettes ($p=0.042$). For those who were never smokers of cigarettes, the effects of carbohydrates and polyunsaturated fats were also independent of each other. Saturated and monounsaturated fates were unrelated to the risk of PD in this sample of men.

B. Potential Neurotoxins:

1. Pesticides and herbicides / years worked on a plantation:

A poster was presented at the 5th International Conference on Progress in Alzheimer's and Parkinson's disease held in Kyoto, Japan March 31 to April 5, 2001. A manuscript prepared from these data was accepted to Archives of Neurology and is in press. (Appendix D) For this manuscript age-adjusted PD incidence was examined by years worked on either a pineapple or sugar plantation, assessed at the baseline examination in 1965 and by pesticide exposure

assessed at the 1971 examination. During follow-up since 1965, 116 men were identified with PD. A dose response effect was observed for years worked on a plantation with age adjusted incidence of PD highest in men who worked more than 10 years on a plantation. The relative risk of PD adjusted for age, pack-years of cigarette smoking, and coffee intake was 1.0 (95% CI = .6-1.6), 1.7 (95% CI = .8-3.7), and 1.9 (95% CI = 1.0-3.5) for men who worked on a plantation 1-10 years, 11-20 years, and more than 20 years compared to men who never did plantation work (p=0.006, test for trend). Years of exposure to pesticides beyond one year appeared to increase the risk of PD although this relationship was not statistically significant. A dose response relationship of PD incidence increasing with increasing years of exposure to pesticides was suggested but not statistically significant (p=0.101 test for trend).

2. Work in sugar cane processing plants:
There were 6844 participants who responded to the question on the 1971 examination regarding work in sugar cane processing. Of 104 cases of PD, 6.73% reported having worked in sugar cane processing compared to 2.72% of non-cases (p=0.026) (the analyses was adjusted for years worked in agriculture on sugarcane plantations). This work was presented at the 2001 American Academy of Neurology Annual Meeting as a platform presentation and is contained in a manuscript by Robert Abbott in press (Appendix C).

3. Other potential toxins with no relationship to risk of PD -

- Exposure to iron: There were 6844 participants who responded to the question on the 1971 examination regarding work related exposure to iron. Of 104 PD cases, 11.5% were exposed compared to 7.6% of 6740 non-cases **(p=0.14).**
- Exposure to paints: There were 6844 participants who responded to the question on the 1971 examination regarding work related exposure to metals. Of 103 PD cases, 9.7% were exposed compared to 14.1% of 6741 non-cases **(p=0.25).** In a logistic regression model adjusting for age, pack years of smoking and coffee consumption, the odds ratio for PD in the exposed versus the unexposed was 0.7 **(p=0.3)**.
- Exposure to dust: There were 6844 participants who responded to the question on the 1971 examination regarding work related exposure to dust. Of 103 PD cases, 9.7% were exposed compared to 7.9% of 6741 non-cases **(p=0.46).** In a logistic regression model adjusting for age, pack years of smoking and coffee consumption, the odds ratio for PD in the exposed versus the unexposed was 1.24 **(p=0.51)**.
- Exposure to sewage: There were 3428 participants who responded to the question on the 1991 examination regarding having worked around sewage. Of 45 PD cases, 6.7% were exposed compared to 5% of 3383 non-cases **(p=0.5).** In a logistic regression model controlling for age, the odds ratio for PD in the exposed versus the unexposed was 1.34 **(p=0.62)**.
- Exposure to metals (general): There were 6844 participants who responded to the question on the 1971 examination regarding work related exposure to metals. Of 103 PD cases, 16.5% were exposed compared to 15.1% of 6741 non-cases **(p=0.5).** In a logistic regression model adjusting for age, pack years of smoking and coffee consumption, the odds ratio for PD in the exposed versus the unexposed was 1.16 **(p=0.6)**.
- Exposure to aluminum: There were 6844 participants who responded to the question on the 1971 examination regarding work related exposure to aluminum. Of 104 PD cases, 3.8% were exposed compared to 4.5% of 6740 non-cases **(p=1).**

- Exposure to mercury: There were 6844 participants who responded to the question on the 1971 examination regarding work related exposure to mercury. Of 104 PD cases, 0% were exposed compared to 0.43% of 6740 non-cases **(p=1).**
- Exposure to steel: There were 6844 participants who responded to the question on the 1971 examination regarding work related exposure to steel. Of 104 PD cases, 8.6% were exposed compared to 6.9% of 6740 non-cases **(p=0.43).**
- Exposure to lead: There were 6844 participants who responded to the question on the 1971 examination regarding work related exposure to lead. Of 104 PD cases, 2.9% were exposed compared to 4.4% of 6740 non-cases **(p=0.63).**
- Occupational exposure to cleaning fluids and solvents: There were 6844 participants who responded to the question on the 1971 examination regarding work related exposure to cleaning fluids and solvents. Of 104 PD cases, 13.5% were exposed compared to 16.0% of 6740 non-cases **(p=.59).**
- Exposure to wood products: There were 6844 participants who responded to the question on the 1971 examination regarding work related exposure to wood products. Of 103 PD cases, 28.2% were exposed compared to 26.4% of 6741 non-cases **(p=0.65).** In a logistic regression model adjusting for age, pack years of smoking and coffee consumption, the odds ratio for PD in the exposed versus the unexposed was 1.16 **(p=0.5)**.
- Exposure to radioactive material: There were 6844 participants who responded to the question on the 1971 examination regarding work related exposure to radioactive material. Of 103 PD cases, 1.9% were exposed compared to 1.7% of 6741 non-cases **(p=0.7).** In a logistic regression model adjusting for age, pack years of smoking and coffee consumption, the odds ratio for PD in the exposed versus the unexposed was 1.24 **(p=0.76)**.
- Exposure to stone and masonry : There were 6844 participants who responded to the question on the 1971 examination regarding work related exposure to stone and masonry. Of 103 PD cases, 20.4% were exposed compared to 19.4% of 6741 non-cases **(p=0.8).** In a logistic regression model adjusting for age, pack years of smoking and coffee consumption, the odds ratio for PD in the exposed versus the unexposed was 1.16 **(p=0.55)**.
- Exposure to welding: There were 6844 participants who responded to the question on the 1971 examination regarding work related exposure to welding. Of 104 PD cases, 6.7% were exposed compared to 7.8% of 6740 non-cases **(p=0.85).**
- Exposure to oils: There were 6844 participants who responded to the question on the 1971 examination regarding work related exposure to oils. Of 103 PD cases, 24.3% were exposed compared to 26.2% of 6741 non-cases **(p=0.74).** In a logistic regression model adjusting for age, pack years of smoking and coffee consumption, the odds ratio for PD in the exposed versus the unexposed was 0.92 **(p=0.72)**.
- Exposure to dyes: There were 6844 participants who responded to the question on the 1971 examination regarding work related exposure to dyes. Of 103 PD cases, 1% were exposed compared to 1.8% of 6741 non-cases **(p=1).** In a logistic regression model adjusting for age, pack years of smoking and coffee consumption, the odds ratio for PD in the exposed versus the unexposed was 0.5 **(p=0.48)**

4. Historical research –specific pesticides used in Hawaii

A list of pesticides in common use on plantations in Hawaii during the years the HHP/HAAS men would likely have been exposed was provided in the 2000 annual report. This information was used in the manuscript entitled "Plantation work and risk of Parkinson's disease in a population based longitudinal study" (Appendix D) and for the poster presentation

entitled "Sugar cane processing is associated with Parkinson's disease in the Honolulu-Asia Aging Study" (see further review of these data in manuscript, Appendix C).

Dr. Sanderson has worked with the HHP/HAAS occupational database to categorize the degree of exposure that cohort members may have had to various pesticides, metals, and solvents based on their reported jobs. This occupational exposure matrix provides a valuable means of assessing the association between occupational exposures and various diseases. Dr. Sanderson is currently expanding the database to include the probability of job exposure to additional metals, chemicals, and noise.

Dr. Sanderson found very high level of manganese in a soil sample from a sugar plantation on Kauai. This finding is important due to reports of parkinsonism in persons exposed to high levels of manganese.[18] Future funding efforts will focus on this issue. Our plan is to follow up on this by taking samples of soil from other areas in the state including as many sites of current or past plantations as possible. Estimates of manganese exposure will then be estimated from these measurements and from existing data on where the men worked and what type of job they had – eg. Outdoor job with heavy machinery that would be associated with heavy dust exposure versus an indoor job not associated with dust exposure.

C. Military service and employment

1. Service during World War II:

There was no association of service during World War II and development of PD. There were 94 men who developed PD out of 6491 (1.45%) who did not serve in World War II compared to 22 men who developed PD among 1479 (1.49%) who did serve in the military during World War II. The difference was not significantly different.

2. Working for the military:

There were 8004 participants who responded to the question on the 1965 baseline examination regarding civilian work for the military. Of 116 PD cases, 6.9% worked for the military compared to 7.9% of 7888 non-cases. In a logistic regression model adjusting for age, pack years of smoking and coffee consumption, the odds ratio for PD in the exposed versus the unexposed was 1 **(p=.98)**.

D. Physical Attributes:

There were several physical attributes measured during midlife that were associated with a higher risk of PD in later life. This may indicate that there are physiological differences that occur early in individuals predisposed to develop PD.

1. Constipation and future PD:

Please see reprint from Neurology 2001;57:456-462 (Appendix E). These data were also presented as a poster at the 5th International Conference on Progress in Alzheimer's and Parkinson's disease held in Kyoto, Japan March 31 to April 5, 2001. There were 6790 men free of PD who responded to the question on the 1971 examination regarding frequency of bowel movements. During the course of follow-up, 96 men developed PD. Age adjusted incidence declined consistently from 18.9 /10,000 person-years in men with <1 bowel movement / day to 3.8/10,000 person-years in those with > 2 bowel movements per day (p=0.005). After adjustment for age, pack-years of smoking, coffee intake, and the use of laxatives, the risk of PD in men with <1 bowel movement per day was 4.5 times greater than the risk in men with > 2 bowel movements per day (p=0.025).

2. Prolonged QT interval and future PD:
Among 8004 Japanese-American men aged 45-65 who received electrocardiograms during the baseline 1965 examination, 134 have been diagnosed with PD to date. The association of corrected QT interval with the incidence of PD was examined using proportional hazards analysis, controlling for age, usual cigarettes smoked per day, and caffeine intake in the prior 24 hours. Relative risks for the development of PD with the lower 25th percentile QT interval as the reference were:1.44 (95% CI 0.93-2.25) for the 25th-75th percentile; 1.24 (95% CI 0.68-2.28) for the 75th-90th percentile; and 2.19 (95% CI 1.21-3.99) for the >90th percentile. Please see manuscript in preparation.(Appendix F)

3. Mid-life adiposity:
The relationships of measurements of body fat (body mass index, triceps skinfold thickness and subscapular skinfold thickness) assessed at the baseline HHP examination in 1965 to incident PD were examined. Among 7990 men who received these adiposity measures in 1965, 137 developed PD over the 30 years of follow-up. After adjusting for cigarette smoking, coffee consumption, physical activity, fat intake and daily caloric intake the relative risk for Parkinson's disease among those in the 4th quartile of triceps skinfold thickness (11-32 mm) compared to those in the 1st quartile (1-5 mm) was 2.8 (95% confidence intervals = 1.4-5.6). These data were presented in a poster at the 5th International Conference on Progress in Alzheimer's and Parkinson's disease held in Kyoto, Japan March 31 to April 5, 2001. A manuscript (Appendix G) has been published in Neurology.

2. Evaluation of epidemiological risk factors for parkinsonism

- Fertilizer and UPDRS: A series of age adjusted logistic regression models were performed examining the association of fertilizer exposure assessed at the 1991 examination with specific items (parkinsonism signs) on the UPDRS (dichotomous outcome score=0 / score >0). For this analysis there were 928 participants who underwent a full neurological examination that included the UPDRS. Cases of PD were removed from this analysis. Odds ratios and p-values are reported in the table below:

UPDRS item	Odds ratio	P-value
Facial expression	1.009	0.97
Finger taps	1.24	0.38
Rapid hand movements	1.21	0.45
Rapid alternating movements	1.17	0.55
Foot agility	0.92	0.73
Body bradykinesia	1.32	0.27
Rigidity	0.84	0.48
Arising from chair	0.79	0.42
Posture	0.85	0.85
Speech	0.93	0.79
Rest tremor	0.64	0.66
Action tremor	0.83	0.48
Postural stability	0.96	0.88
Gait	1.03	0.9

In a second analysis using logistic regression, exposure to fertilizer was examined as a predictor of the total number of UPDRS signs present. There was no association of fertilizer exposure with number of parkinsonism signs present (p=0.91).

- Years worked on plantation and UPDRS: The same 928 individuals without PD who had a full neurological examination with UPDRS were used to analyze the association of years worked on a plantation with individual items on the UPDRS. In a series of logistic regression analyses, years worked on a plantation was positively associated with limb rigidity, slowing of rapid alternating movements, and stooped posture (p=0.08, 0.08, and 0.04 respectively). When adjusted for age these associations were non-significant. There was no association of years worked on a plantation with the number of parkinsonism signs found on the UPDRS or with the total sum of the UPDRS scores.
- Years exposed to pesticides and UPDRS: In a series of logistic regression analyses that included 928 participants, years exposed to pesticides was not related to any of the individual items from the UPDRS. Similarly there was no relationship between years exposed to pesticides and the number of parkinsonism signs present or with the total sum of the UPDRS scores.
- Cigarette smoking and UPDRS: In a series of logistic regression models adjusted for age, and coffee consumption, that included 928 participants, pack-years of cigarette smoking was positively related to slowed finger taps (p=0.07), slow rise from chair (p=0.049), slow gait (p=0.09), and body bradykinesia (p=0.047). Note that this is the opposite of what would be expected given the inverse relationship of smoking with PD incidence. However, there was no relationship between pack years smoked and number of parkinsonism signs present or total sum of the UPDRS scores.
- Coffee drinking and UPDRS: In a series of univariate logistic regression models that included 928 participants, coffee intake at the 1965 examination was inversely associated with slowed rapid alternating movements (p=0.07) and slowed foot tapping (p=0.09) from the UPDRS. These findings became non-significant with adjustment for age. There was no association of coffee consumption with number of parkinsonism signs present or with the total sum of the UPDRS score.
- Military services and UPDRS: In a series of univariate logistic models that included 928 participants, service in the military during World War II was significantly inversely related to all items on the UPDRS except rest tremor and action tremor. However, this is misleading because those who served in the military during World War II were the younger members of the cohort. When the logistic models were adjusted for age, World War II military service was only associated with postural instability (inverse) (p=0.047). This relationship was only of borderline significance when adjusted for age, coffee, and pack years of smoking (p=0.059). There was no association of World War II military service with the number of parkinsonism signs present or the total sum of the UPDRS score.

3. Neuropathology Component:

A. Evaluation of myelin abnormalities

As of September 30, 2002 there have been 490 brains acquired. A full set of histologic sections of nine separate blocks of cerebral white matter stained with hematoxylin and eosin, luxol fast

blue/Bodian and anti-amyloid beta protein have been prepared for 188 cases. Neuropathologic studies are ongoing on cerebral white matter from these cases. The studies involve quantitative assessment of myelin loss from six of the standard sections of white matter with a photometric method developed in the course of the investigation. The myelin loss is then correlated with other variables including astrocytic, vascular, and ischemic changes that may accompany the myelin loss, atherosclerosis of the circle of Willis, cognitive status, gait and mobility disturbances and exposures to potentially hazardous environmental agents. Photometric analysis of 40 cases has been completed and preliminary statistical and correlative analyses have been performed. Within individuals all of the white matter readings are correlated to each other and correlation coefficients are in the range of 0.3 to 0.7. There were strong correlations between white matter photometric readings and the indicators for neocortical and hippocampal neuritic plaque densities, and for the average neocortical GFAP tissue level. No significant correlations were found for neocortical tangles, lacunes, large vessel infarcts, mantle thickness, or brain weight.

White matter pallor was strongly predictive of low cognitive abilities screening instrument (CASI) score during life after controlling for neuritic plaques, neurofibrillary tangles, and lacunes. Adding cortical mantle thickness reduced the strength of the association to a marginal level.

B. Evaluation of Lewy bodies

Microscopic evaluations of Lewy bodies in the HAAS brains are ongoing and have been completed on over 350 cases. H&E stained sections of pons and mid-brain are examined for the presence of Lewy bodies in the locus ceruleus and substantia nigra respectively. Those with Lewy bodies in the brainstem are examined for cortical Lewy bodies with alpha synuclein stains. Analyses have been conducted both using all Lewy body cases and using only those that did not have PD during life (incidental Lewy body cases). The analyses include:

1. Reaction time and Lewy bodies

Reaction time may be of interest in early detection of PD. Patients with PD have slow scores on tests of both the simple and choice reaction time compared to age matched normal controls. This is thought to be due to a combination of deficits in both attention control and pre-programming of movement. The effect on reaction time has been shown to be present in early and untreated Parkinson's disease. Additionally, reaction time has been shown to correlate with nigrostriatal degeneration as measured by [123I] B-CIT SPECT in patients with PD.[19] The aim of these ongoing analyses is to correlate the Reaction Time (RT) as measured by a standard computer-administered reaction time test with the presence of incidental Lewy Bodies. The hypothesis is that slower RT will correlate with the presence of incidental Lewy bodies in the pons or substantia nigra. Future analyses will evaluate the relationship between reaction time and striatal dopamine levels and neuron counts in the substantia nigra. It is hoped that additional incident cases of PD will add sufficient power to evaluate the relationship between reaction time and future PD.

THE REACTION TIME (RT) TEST: The RT test as administered to HAAS participants consists of two parts, the simple RT task and the choice RT task, and is performed with a laptop computer.[20] Two stimulus figures are used for both tasks, the left- and right- pointing arrowheads. The simple RT task has two segments, the left and right, both with 16 trials. Before the start of each segment the subject is told which stimulus figure will be shown and is asked to use the left index finger to press the Z key on the keyboard for the left trials, or to use

the right finger to press the ?/ key for the right trials. For the choice RT task 16 left and 16 right trials are presented in pseudo-random order, with four left and four right trials in each block of eight trials. The subject is told that the direction of the arrow will be unpredictable and is asked to respond with the hand indicated by the arrow. The simple RT is administered before the choice RT. Each task begins with an instruction screen that shows the stimulus figures and the expected responses. A press of the space bar terminates the instruction screen and starts the first trial.

For this analysis, there were 96 brains from men who had reaction time testing before death. Of these, 8 had Lewy bodies in either the substantia nigra or the locus ceruleus and no history of Parkinson's disease during life (incidental Lewy bodies). The table below shows the per cent of men with incidental Lewy bodies found in the pons or substantia nigra according to quartile of simple and choice total reaction times. Reaction times are reported in milliseconds (ms).

		Quartile of total reaction time				
Test	Response	1st	2nd	3rd	4th	p-value*
Simple	Median	0.0 (0/24)#	4.2 (1/24)	12.5 (3/24)	16.7 (4/24)	0.037
Choice	Median	0.0 (0/24)	4.2 (1/24	20.8 (5/24)	8.3 (2/24)	0.181

* test for trend after adjusting for age at the time of reaction time testing
# Cases of incidental Lewy bodies / sample size

The data indicate that incidental Lewy bodies are more likely to occur in men with longer simple reaction times during life. The effect is most pronounced in the simple reaction time test which primarily measures speed of physical reaction. These data will be presented in a poster at the Seventh International Congress of Parkinson's Disease and Movement Disorders to be held in Miami, Florida November 10-14, 2002 (Appendix H).

Future analyses will examine the association of reaction time with striatal dopamine levels and neuron counts in the substantia nigra. It is hoped that enough incident cases of Parkinson's disease will eventually be identified in the HHP/HAAS cohort that the reaction time test can be examined as a predictor of clinical PD.

2. Olfaction and Lewy bodies

An analysis examines the association of impaired olfaction with incidental Lewy bodies in the brainstem. Impaired olfaction has been reported to be an early finding in Parkinson's disease. It is hypothesized that neurotoxins that affect the dopamine producing neurons may gain entry into the brain through the olfactory system. Our aim was to determine if olfaction is impaired

during life in individuals found at autopsy to have incidental Lewy bodies in their locus ceruleus or substantia nigra. Olfaction was measured during the 4th and 5th examinations of the HAAS (1991 and 1994) using the University of Pennsylvania smell identification test. Among 92 brains examined from participants who had a smell test at exam 5, there is a significant association of impaired smell with the presence of Lewy bodies after age adjustment (p=0.0085).These data were (Appendix I) presented by Dr. Ross at the annual meeting of the American Academy of Neurology in San Diego, CA April 22, 2000.

3. Smoking, Coffee drinking and Lewy bodies

One abstract (Appendix J) was presented by Dr. Ross at the annual meeting of the American Academy of Neurology in Toronto, Ontario April 22 entitled "Relationship of smoking and coffee consumption to presence of Lewy bodies in the brainstem." The objective of this analysis was to determine the association of cigarette and coffee use with the presence of Lewy bodies in the substantia nigra or locus ceruleus . The population consisted of 260 brains of deceased HHP/HAAS cohort members. H&E stained sections of locus ceruleus and substantia nigra were examined for Lewy bodies. Cigarette smoking and coffee drinking were assessed at full cohort exams in 1965 and 1971. Lewy bodies were found in 43 (16.5%) brains. There was an increase in the frequency of Lewy bodies with age ranging from 4.7% of brains in the 70-74 year old age group to 34.9% in the 85-89 year old age group. Logistic regression was used to calculate the age adjusted odds ratio for having Lewy bodies by pack years of smoking. There was no significant association of smoking with Lewy bodies. Additionally, there was no association of coffee drinking with Lewy bodies although a dose response pattern was evident with heavy coffee drinkers having the lowest risk of having Lewy bodies.

4. Cognitive function and Lewy bodies

Clinicopathological data from 285 autopsies were analyzed to determine the association of four primary neuropathologic processes (microvascular lesions, Alzheimer lesions, hippocampal sclerosis, and cortical Lewy bodies) with clinical dementia. Please see page proofs of manuscript by Dr. Lon White accepted for publication in the Annals of the New York Academy of Sciences (Appendix K). When Lewy bodies were limited to the substantia nigra or locus ceruleus, no association with dementia was noted. In contrast, when Lewy bodies were found in any of the neocortical regions, there was a substantial association with dementia. In a logistic regression model adjusted for age, education, and the other neuropathological lesions, the relative risk of having definite or probable dementia was 2.17 (95% confidence interval = 0.83-5.64) for those having at least one cortical Lewy body compared to those with none.

5. The relationship of Lewy bodies and Alzheimer lesions

There is clinical overlap between Alzheimer's disease and dementia related to Parkinson's disease and the neuritic plaques and neurofibrillary tangles of AD often coexist with cortical Lewy bodies. These findings have led some to hypothesize a common pathophysiological process underlying these two conditions. It is not clear, however, whether the lesions, both of which are common in the elderly, occur together more commonly than what would be expected by chance alone. For this analysis 324 brains were divided into three mutually exclusive groups – those without Lewy bodies (N=277), those with Lewy bodies confined to the substantia nigra or locus ceruleus (N=9), and those with cortical Lewy bodies (N=38). The three groups has similar frequency of Alzheimer lesions. Age adjusted odds ratios for having neuritic plaques in those with brainstem only Lewy bodies was 0.9 (95% confidence interval = 0.23-3.5) and for those with cortical Lewy bodies was 0.83 (95% confidence interval = 0.44-1.6) compared to the

group with no Lewy bodies. Odds ratios for having neurofibrillary tangles were 1.02 (95% confidence interval = 0.24-4.25) and 0.98 (95% confidence interval = 0.46-2.09) respectively. Cortical Lewy bodies were associated with neuronal loss in the nucleus basalis and amygdala. Age adjusted odds for neuronal loss in these areas for the cortical Lewy body group compared to the no Lewy body group was 3.6 (95% confidence interval = 0.1.7-7.6) and 2.4 (95% confidence interval = 1.2-4.6) respectively. These data suggest that the presence of brainstem or cortical Lewy bodies does not predispose an individual to have Alzheimer type pathology. The clinical overlap of the dementia syndromes associated with Alzheimer type pathology and Lewy bodies may be related in part to the common brain regions damaged by processes leading to these lesions. This work was presented as a platform presentation at the Eighth International Conference of Alzheimer's disease and Related Disorders in Stockholm, Sweden, July 23, 2002 (Appendix L).

6. The relationship of extrapyramidal signs to incidental Lewy bodies

A manuscript by study co-investigator and biostatistician, Dr. Rob Abbott, is in preparation regarding the extrapyramidal features most associated with incidental Lewy bodies at autopsy. The population consists of 134 autopsied men without Parkinson's disease, aged 76 to 97 years at the time of death, who were examined within approximately three years of death with the Unified Parkinson's Disease Rating Scale (UPDRS). The set of clinical features from the UPDRS most strongly related to the presence of Lewy-bodies included slow "rapid alternating movements of hands", slow "hand movements", "rigidity", "body bradykinesia and hypkinesia", "action or postural tremor of hands", and "tremor at rest". In men who had 1 or fewer of these features, there were no cases of incidental Lewy bodies (0/11). As the number of features increased, the percent of men with incidental Lewy bodies rose significantly to 31.6% (6/19) in men with 5 or more clinical features ($p<0.004$). (Appendix M)

C. Glial fibrillary acidic protein assays

As stated in previous reports, NIOSH neurotoxicologists have developed a procedure using enzyme linked immunosorbant assay techniques to quantify glial fibrillary acidic protein (GFAP) and have found that GFAP is elevated in the nigral-striatal regions after exposure to neurotoxicants such as MPTP.[21-23]

GFAP is produced by astrocytes and is the major constituent of glial filaments that accumulate in response to central nervous system injury in a process called astrogliosis or reactive gliosis.[22,23] Reactive gliosis can be induced by a number of insults to the brain including physical damage, disease, or chemicals. Glial proliferation occurs in Alzheimer's disease and though the mechanism is unknown, it is postulated to be related to inflammatory processes. Inflammation has also been hypothesized to play a role in the pathophysiology of Parkinson's disease and this is currently an important area of research.

Investigators in Honolulu and at NIOSH have competed a case-control study using frozen tissue blocks from cortical regions in ten Alzheimer disease brains, ten age matched controls and ten young controls. GFAP was measured at the NIOSH labs in Morgantown West Virginia. GFAP was measured in four neocortical areas from each brain – frontal, temporal,

parietal and occipital cortices. GFAP levels were significantly higher in the AD brains in temporal, parietal, and occipital lobes, with the greatest difference being in the temporal lobe. These results have been written into a manuscript that has been accepted to Acta Neurologica Scandinavica (see appendix N)

Investigators in Honolulu and at NIOSH have also examined the association of GFAP levels measured in four neocortical areas – frontal, temporal, parietal and occipital cortices with cognitive performance test scores, dementia diagnostic categories, as well as neurofibrillary tangle and neuritic plaque counts in 204 brains in the HAAS brain bank. Cognitive function as measured by the Cognitive Abilities Screening Instrument was inversely associated with GFAP levels in the temporal, parietal, and occipital lobes, but not the frontal lobe. These relationships remained significant after adjustment for neuritic plaque and neurofibrillary tangle counts suggesting that other factors in addition to Alzheimer pathology were contributing to brain damage as measured by GFAP. (see manuscript in preparation, appendix O)

D. Brain neurotoxin (organochlorine) assay:

The role for pesticide exposure in IPD has also been supported by a reports of the detection of significantly higher levels of organochlorine compounds in brains of PD cases compared to AD cases or normal controls[24,25] Accordingly, one of the specific aims in our original statement of work was to determine levels of organochlorine compounds in the brains of those autopsy cases in our brain bank with the highest exposure history to pesticides. For the original pilot work included in the statement of work, fifteen cases were selected from the HAAS brain bank with the highest exposure to pesticides during life by self reported years worked on a pineapple plantation (organochlorines were used extensively on pineapple plantations in Hawaii in the post World War II years) and by self reported exposure to pesticides. Two frozen blocks of cortex with white matter were sent from each of the fifteen brains selected, one from the occipital pole and the other from the frontal pole. Organochlorine pesticide and lipid analysis were performed on each of these at the Analytical and Chemical Sciences lab, Research Triangle Institute, Research Triangle Park, NC under the direction of Dr. E. D. Pellizzari. Attached is a spreadsheet with these data (Table, Appendix P).

The sample numbers represent samples from occipital lobe and frontal lobe from brains of 15 subjects. For example, samples 2160A-27 and 2160A-7 represent frontal and occipital samples from the same brain. The prefix Du for some of the samples means duplicate measurements were performed on one sample as an assessment of reliability. Values in the table are final concentrations in parts per billion (ng/gm; ug/kg). MB-# are the methods blanks and MC-# the methods controls. QL is the quantifiable limit defined as the lowest detectable level that the lab feels confident reporting. It is determined by the formula: QL for the chemical times 0.08 divided by the sample mass. The QL varies from sample to sample due to the variation in size of sample used. Therefore, smaller samples would be associated with higher QLs and more nondetectable (nd) results. Importantly, the sample weights from the HAAS brain tissue (around 80 mg) were judged by Dr. Pellizzari and his lab to be acceptable. Several conclusions can be drawn from these data:

1. The matching pairs of samples from the frontal and occipital lobes have values that are amazingly close (according to Dr. Pelazarri the values are as close as would be expected if the same sample were assayed twice) suggesting that over time levels of these

compounds are distributed fairly evenly throughout the cortical tissues. Therefore, it will be safe to use just one sample per brain for future levels.

2. Several samples have levels of one or more organochlorine compounds higher than 10 ppb, levels that would be considered significantly high if found in the blood. DDE, g-chlordane, and methoxy are especially prominent.
3. Regarding use of the compounds: DDT (metabolized in the body to DDE and DDD) was in very common use in the agricultural, industrial, and home settings in the state of Hawaii against a variety of pests including mosquitoes. Chlordane (termite control) and mirex (ant control) were commonly used around homes. Methoxy, dieldrin, aldrin, and heptachlor had more specific agricultural / industrial uses.
4. It is safe to say that detectable levels of these substances in any individual brain reflect exposure to those compounds during the time that the compounds were in use. These chemicals remain detectable in tissues for many years. In some cases there are more than 30 years between the exposure (ie to plantation work or self report of exposure to pesticides) and death. Therefore, these data can provide a quantifiable exposure measurement that, along with occupational data, will allow calculation of the age at the time of exposure.

Having shown that it is feasible to obtain detectable levels of organochlorine compounds in the brains of deceased HHP / HAAS participants with a history of plantation work or pesticide exposure, and that plantation work in Hawaii is associated with a higher risk of PD in the HHP / HAAS cohort we are now prepared to perform organochlorine measurements on all brains for which we have frozen tissue in our brain bank. This will allow us to examine the association of high levels of organochlorines with clinical PD (N=17 of 335 brains with complete microscopic evaluations to date). Key to this work, however, is our ability to go beyond previous work to examine the association of brain organochlorine levels with pathological markers or determinants of PD and other degenerative brain conditions.

Existing neuropathological data will enable us to examine the relationship of organochlorine levels with Lewy bodies present in the substantia nigra or locus ceruleus (N=43 of 335 brains with complete microscopic evaluations to date) of brains from deceased HAAS participants. Furthermore, we are now performing dopaminergic neuron counts on single sections through the substantia nigra and dopamine / dopamine metabolite levels on frozen samples of the caudate nucleus and putamen from all brains in the HAAS brain bank as part of a National Institute of Neurological Disorders and Stroke funded project. This presents a unique opportunity unlikely to be available anywhere else in the world to directly examine the relationship between organochlorine levels and neuropathological markers of PD within the same brain. The demonstration of high brain levels of organochlorines with low numbers of substantia nigra dopamine neurons and/or low levels of striatal dopamine would provide compelling evidence for a direct toxic effect of organochlorines on the nigrostriatal system in humans.

Another component of the existing DOD supported project is a study of glial fibrillary acidic protein (GFAP) brain levels in Alzheimer's disease and Parkinson's disease. GFAP is produced by astrocytes and is the major constituent of glial filaments that accumulate in response to central nervous system injury in a process called astrogliosis or reactive gliosis.[23,26] Reactive gliosis can be induced by a number of insults to the brain including physical damage, disease, or chemicals. Glial proliferation occurs in Alzheimer's disease and though the mechanism is unknown, it is postulated to be related to inflammatory processes. Inflammation

has also been hypothesized to play a role in the pathophysiology of Parkinson's disease and this is currently an important area of research.

Work is currently underway to perform the GFAP assay on frozen samples of caudate and putamen and to examine associations of GFAP levels in these regions with Parkinson's disease and parkinsonism. The availability of these GFAP levels presents an excellent opportunity to examine the association of brain organochlorine levels with this sensitive measure of brain injury.

Although the cognitive effects of organochlorine compounds are not as generally recognized as the effects on the motor system, it is reasonable to hypothesize that organochlorine toxicity may cause cognitive and behavioral dysfunction. One of the primary goals of the HAAS is to identify and sub-classify all cases of dementia in the cohort. All participants receive cognitive screening during HAAS examinations using the cognitive abilities screening instrument (CASI). Neuropathological efforts include a comprehensive standardized gross and microscopic examination of the brain that includes neurofibrillary tangle and neuritic plaque counts in multiple cortical and hippocampal regions. The availability of these data would allow us to examine the association of brain organochlorine levels with clinical endpoints including CASI score as a general measure of cognitive function and dementia diagnosis; and with neuropathological endpoints including neurofibrillary tangles and neuritic plaques.

4. Genetics Study:

A subcontract was established with Stanford University to use DNA samples from approximately 117 Parkinson's disease cases and 240 controls without Parkinson's disease matched for age for genotyping for the following five polymorphisms. In the tables below, PD case refers to DNA from subjects with Parkinson's disease while Incidental LB refers to DNA from deceased subjects without a history of Parkinson's disease whose brains have Lewy bodies in the substantia nigra or locus ceruleus.

1. CYP2D6 HhaI polymorphism in exon 6. CYP2D6 is a subfamily of the cytochrome P-450 enzyme system in the liver. This enzyme system catalyzes breakdown of many potential environmental neurotoxins and medications. Mutant alleles of the gene lead to poor or slow metabolism of debrisoquine and similar medications.

CYP2D6

Genotype frequency for PD Case plus Incidental LB

	N	C/C	N (%)	C/T	N (%)	T/T	N (%)	N/D	N (%)
Case	117	83	70.9%	27	23.1%	5	4.3%	2	1.7%
Control	240	167	69.6%	63	26.3%	8	3.3%	2	0.8%

Genotype frequency for PD Case

	N	C/C	N (%)	C/T	N (%)	T/T	N (%)	N/D	N (%)
Case	89	67	75.3%	18	20.2%	3	3.4%	1	1.1%
Control	184	127	69.0%	49	26.6%	7	3.8%	1	0.5%

Genotype frequency for Incidental LB

	N	C/C	N (%)	C/T	N (%)	T/T	N (%)	N/D	N (%)
Case	28	16	57.1%	9	32.1%	2	7.1%	1	3.6%
Control	56	40	71.4%	14	25.0%	1	1.8%	1	1.8%

Allele frequency for PD Case plus incidental LB

	N	C	N (%)	T	N (%)	N/D	N (%)
Case	234	193	82.5%	37	15.8%	4	1.7%
Control	480	397	82.7%	79	16.5%	4	0.8%

Allele frequency for PD Case

	N	C	N (%)	T	N (%)	N/D	N (%)
Case	178	152	85.4%	24	13.5%	2	1.1%
Control	368	303	82.3%	63	17.1%	2	0.5%

Allele frequency for Incidental LB

	N	C	N (%)	T	N (%)	N/D	N (%)
Case	56	41	73.2%	13	23.2%	2	3.6%
Control	112	94	83.9%	16	14.3%	2	1.8%

2. CYP1A2 promoter polymorphism. This is a genetic polymorphism in the 5′-flanking region of human CYP1A2 gene that has a major effect on the elimination of caffeine [12]. The purpose of this analysis is to investigate a possible genetic explanation for our recent finding that caffeine consumption is inversely associated with PD incidence.[27]

CYP1A2

Genotype frequency for PD Case plus incidental LB

	N	A/A	N (%)	A/G	N (%)	G/G	N (%)	N/D	N (%)
Case	117	6	5.1%	46	39.3%	63	53.8%	2	1.7%
Control	240	17	7.1%	85	35.4%	138	57.5%	0	0.0%

Genotype frequency for PD Case

	N	A/A	N (%)	A/G	N (%)	G/G	N (%)	N/D	N (%)
Case	89	4	4.5%	33	37.1%	51	57.3%	1	1.1%
Control	184	14	7.6%	64	34.8%	106	57.6%	0	0.0%

Genotype frequency for Incidental LB

	N	A/A	N (%)	A/G	N (%)	G/G	N (%)	N/D	N (%)
Case	28	2	7.1%	13	46.4%	12	42.9%	1	3.6%
Control	56	3	5.4%	21	37.5%	32	57.1%	0	0.0%

Allele frequency for PD Case plus Incidental LB

	N	A	N (%)	G	N (%)	N/D	N (%)
Case	234	58	24.8%	172	73.5%	4	1.7%
Control	480	119	24.8%	361	75.2%	0	0.0%

Allele frequency for PD Case

	N	A	N (%)	G	N (%)	N/D	N (%)
Case	178	41	23.0%	135	75.8%	2	1.1%
Control	368	92	25.0%	276	75.0%	0	0.0%

Allele frequency for Incidental LB

	N	A	N (%)	G	N (%)	N/D	N (%)
Case	56	17	30.4%	37	66.1%	2	3.6%
Control	112	27	24.1%	85	75.9%	0	0.0%

3. Dopamine transporter (DAT) 1215 A/G. This is a polymorphism in exon 9 of the dopamine transporter gene.[10] In a study of Japanese subjects, this polymorphism was found to be significantly less frequent among PD cases compared to controls.

DAT1215

Genotype frequency for PD Case plus Incidental LB

	N	A/A	N (%)	A/G	N (%)	G/G	N (%)	N/D	N (%)
Case	117	97	82.9%	16	13.7%	2	1.7%	2	1.7%
Control	240	201	83.8%	35	14.6%	3	1.3%	1	0.4%

Genotype frequency for PD Case

	N	A/A	N (%)	A/G	N (%)	G/G	N (%)	N/D	N (%)
Case	89	78	87.6%	9	10.1%	1	1.1%	1	1.1%
Control	184	153	83.2%	28	15.2%	2	1.1%	1	0.5%

Genotype frequency for Incidental LB

	N	A/A	N (%)	A/G	N (%)	G/G	N (%)	N/D	N (%)
Case	28	19	67.9%	7	25.0%	1	3.6%	1	3.6%
Control	56	48	85.7%	7	12.5%	1	1.8%	0	0.0%

Allele frequency for PD Case plus incidental LB

	N	A	N (%)	G	N (%)	N/D	N (%)
Case	234	210	89.7%	20	8.5%	4	1.7%

Control	480	437	91.0%	41	8.5%	2	0.4%

Allele frequency for PD Case

	N	A	N (%)	G	N (%)	N/D	N (%)
Case	178	165	92.7%	11	6.2%	2	1.1%
Control	368	334	90.8%	32	8.7%	2	0.5%

Allele frequency for Incidental LB

	N	A	N (%)	G	N (%)	N/D	N (%)
Case	56	45	80.4%	9	16.1%	2	3.6%
Control	112	103	92.0%	9	8.0%	0	0.0%

4. Parkin Arg366Trp [13] This is a polymorphism in exon 10 of the parkin gene that has been found to be significantly lower in PD cases compared to controls.

PARKIN

Genotype frequency for PD Case plus Incidental LB

	N	C/C	N (%)	C/T	N (%)	T/T	N (%)	N/D	N (%)
Case	117	113	96.6%	3	2.6%	0	0.0%	1	0.9%
Control	240	238	99.2%	2	0.8%	0	0.0%	0	0.0%

Genotype frequency for PD Case

	N	C/C	N (%)	C/T	N (%)	T/T	N (%)	N/D	N (%)
Case	89	87	97.8%	2	2.2%	0	0.0%	0	0.0%
Control	184	182	98.9%	2	1.1%	0	0.0%	0	0.0%

Genotype frequency for Incidental LB

	N	C/C	N (%)	C/T	N (%)	T/T	N (%)	N/D	N (%)
Case	28	26	92.9%	1	3.6%	0	0.0%	1	3.6%
Control	56	56	#####	0	0.0%	0	0.0%	0	0.0%

Allele frequency for PD Case plus Incidental LB

	N	C	N (%)	T	N (%)	N/D	N (%)
Case	234	229	97.9%	3	1.3%	2	0.9%
Control	480	478	99.6%	2	0.4%	0	0.0%

Allele frequency for PD Case

	N	C	N (%)	T	N (%)	N/D	N (%)
Case	178	176	98.9%	2	1.1%	0	0.0%
Control	368	366	99.5%	2	0.5%	0	0.0%

Allele frequency for incidental LB

	N	C	N (%)	T	N (%)	N/D	N (%)
Case	56	53	94.6%	1	1.8%	2	3.6%
Control	112	112	#####	0	0.0%	0	0.0%

5. Adenosine A2A Receptor 1083 T/C polymorphism [14] This is a polymorphism in exon 2 of the human A2A human adenosine receptor gene. Caffeine, an adenosine A2A receptor blocker, has been associated with a decreased risk of developing Parkinson's disease. It is hypothesized that polymorphisms of the A2A receptor gene may alter risk for Parkinson's disease.

ADENOSINE A2A RECEPTOR

Genotype frequency for PD Case plus Incidental LB

	N	C/C	N (%)	C/T	N (%)	T/T	N (%)	N/D	N (%)
Case	117	25	21.4%	56	47.9%	34	29.1%	2	1.7%
Control	240	55	22.9%	111	46.3%	72	30.0%	2	0.8%

Genotype frequency for PD Case

	N	C/C	N (%)	C/T	N (%)	T/T	N (%)	N/D	N (%)
Case	89	20	22.5%	44	49.4%	24	27.0%	1	1.1%
Control	184	38	20.7%	84	45.7%	61	33.2%	1	0.5%

Genotype frequency for Incidental LB

	N	C/C	N (%)	C/T	N (%)	T/T	N (%)	N/D	N (%)
Case	28	5	17.9%	12	42.9%	10	35.7%	1	3.6%
Control	56	17	30.4%	27	48.2%	11	19.6%	1	1.8%

Allele frequency for PD Case plus Incidental LB

	N	C	N (%)	T	N (%)	N/D	N (%)
Case	234	106	45.3%	124	53.0%	4	1.7%
Control	480	221	46.0%	255	53.1%	4	0.8%

Allele frequency for PD Case

	N	C	N (%)	T	N (%)	N/D	N (%)
Case	178	84	47.2%	92	51.7%	2	1.1%
Control	368	160	43.5%	206	56.0%	2	0.5%

Allele frequency for Incidental LB

	N	C	N (%)	T	N (%)	N/D	N (%)
Case	56	22	39.3%	32	57.1%	2	3.6%

Control	112	61	54.5%	49	43.8%	2	1.8%

6. Paraoxonase 1 Met-Leu 54 polymorphism [15] Two polymorphisms of the paraoxonase 1 gene affect the hydrolysis of toxic oxons and are thought to increase the toxic effects of environmental chemicals as they relate to the etiology of PD. One report has shown an association of the Met-Leu 54 polymorphism with PD.

PARAOXONASE 1 MET-LEU POLYMORPHISM

Genotype frequency for PD Case plus Incidental LB

	N	T/T	N (%)	A/T	N (%)	A/A	N (%)	N/D	N (%)
Case	116	96	82.8%	19	16.4%	1	0.8%	0	0%
Control	240	212	88.3%	26	10.8%	2	0.8%	0	0.0%

Genotype frequency for PD Case

	N	T/T	N (%)	A/T	N (%)	A/A	N (%)	N/D	N (%)
Case	88	72	81.8%	16	18.2%	0	0%	0	0%
Control	184	163	88.6%	19	10.3%	2	1.1%	0	0.0%

Genotype frequency for Incidental LB

	N	T/T	N (%)	A/T	N (%)	A/A	N (%)	N/D	N (%)
Case	28	24	85.7%	3	10.7%	1	3.8%	0	0%
Control	56	49	87.5%	7	12.5%	0	0%	0	0%

7. Dopamine receptor D2 *Taq*IA polymorphisms affect risk of developing motor fluctuations in PD.[17] Individuals carrying the A1 allele of the D2 TaqIA polymorphism have been reported to have reduced striatal D2 dopamine receptor numbers. A recent report [17] found an excess of the A1A1 genotype in PD patients with motor fluctuations. Here, we investigated an association of the D2 *Taq*IA polymorphism with PD

DOPAMINE RECEPTOR D2 *Taq*IA POLYMORPHISM

Genotype frequency for PD Case plus Incidental LB

	N	A2/A2	N (%)	A2/A1	N (%)	A1/A1	N (%)	N/D	N (%)
Case	116	54	46.6%	40	34.5%	18	15.5%	4	3.4%
Control	240	102	42.5%	100	41.7%	36	15.0%	2	0.8%

Genotype frequency for PD Case

	N	A2/A2	N (%)	A2/A1	N (%)	A1/A1	N (%)	N/D	N (%)
Case	88	39	44.3%	33	37.5%	13	14.8%	3	3.4%
Control	184	79	42.9%	75	40.8%	29	15.8%	1	0.5%

Genotype frequency for Incidental LB

	N	A2/A2	N (%)	A2/A1	N (%)	A1/A1	N (%)	N/D	N (%)
Case	28	15	53.6%	7	25%	5	17.9%	1	3.6%
Control	56	23	41.1%	25	44.6%	7	12.5%	1	1.8%

8. Vesicular monoamine transporter 2 (VMAT2) polymorphism may be associated with abnormality of the VMAT 2 protein that is responsible for packaging and transport of monoamines within the cell. Disruption of this system could cause toxic levels of monoamines resulting in cell death.

VESICULAR MONOAMINE TRANSPORTER 2 POLYMORPHISM

Genotype frequency for PD Case plus Incidental LB

	N	T/T	N (%)	T/G	N (%)	G/G	N (%)	N/D	N (%)
Case	116	93	80.2%	21	18.1%	1	0.8%	1	0.8%
Control	240	207	86.3%	32	13.3%	1	0.4%	0	0.0%

Genotype frequency for PD Case

	N	T/T	N (%)	T/G	N (%)	G/G	N (%)	N/D	N (%)
Case	88	70	79.5%	18	20.5%	0	0%	0	0%
Control	184	159	86.4%	24	13.0%	1	0.5%	0	0.5%

Genotype frequency for Incidental LB

	N	T/T	N (%)	T/G	N (%)	G/G	N (%)	N/D	N (%)
Case	28	23	82.1%	3	10.7%	1	3.6%	1	3.6%
Control	56	48	85.7%	8	14.3%	0	0%	0	0%

None of the polymorphisms studied thus far have shown a significant relationship with Parkinson's disease or with incidental Lewy bodies in the HHP/HAAS cohort.

KEY RESEARCH ACCOMPLISHMENTS:

- Coffee drinking as well as caffeine consumption from non-coffee sources measured prospectively at two separate HHP examinations are associated with a decreased risk of developing Parkinson's disease up to 30 years later
- Although statistically non-significant, high levels of coffee consumption are associated with a lower risk of incidental Lewy bodies suggesting that coffee drinking may be related to the mechanism(s) leading to the formation of Lewy bodies.
- There is no consistent relationship between cigarette smoking and the presence of brainstem Lewy bodies.
- The set of clinical features from the Unified Parkinson's Disease Rating Scale most strongly related to the presence of incidental Lewy-bodies (Lewy bodies in subjects without

Parkinson's disease) include slow "rapid alternating movements of hands", slow "hand movements", "action or postural tremor of hands", and "tremor at rest".

- Glial fibrillary acidic protein is elevated in the cortex of brains of patients with Alzheimer's disease compared to age matched and young controls. This is most evident in the temporal lobe.
- GFAP levels in three neocortical regions measured after death are inversely associated with cognitive function during life independent of neuropathologic lesions of Alzheimer's disease.
- Working in sugar cane processing is associated with increased risk of PD.
- Years working on a plantation (significant) and years exposed to pesticides (non-significant) are associated with PD
- Prolonged QT interval on electrocardiogram is associated with the future development of PD
- Impaired olfaction measured approximately three years prior to death is associated with the presence of incidental Lewy bodies in the substantia nigra or locus ceruleus.
- Service in the military during World War II and working for the military were not associated with increased risk of PD.
- Exposure to metals was not associated with increased risk of PD in the HAAS cohort.
- Occupational exposure to welding was not associated with increased risk of PD in the HAAS cohort.
- Constipation measured as bowel movement frequency is associated with the future development of PD. Manuscript published in Neurology [28](Appendix E)
- Increased triceps skinfold thickness is associated with the future development of Parkinson's disease.
- The presence of brainstem or cortical Lewy bodies does not predispose an individual to have Alzheimer type pathology. The clinical overlap of the dementia syndromes associated with Alzheimer type pathology and Lewy bodies may be related in part to the common brain regions damaged by processes leading to these lesions.
- Consumption of fruit but not vitamin C is associated with increased PD risk after adjustment for other PD risk factors.
- Dietary carbohydrate intake is associated with increased risk of PD.
- Dietary polyunsaturated fats are associated with lower risk of PD
- There is no relationship between risk of PD and intake of total calories, saturated and monounsaturated fats, protein, niacin, riboflavin, beta carotene, vitamins A,B,C and E, and dietary cholesterol, cobalamin, and pantothenic acid.
- There was no relationship of fertilizer exposure, years worked on a plantation, cigarette smoking, coffee consumption, or service in the military with number of parkinsonism signs present or with the total sum of the UPDRS score among elderly participants in the HAAS without PD.
- The extrapyramidal signs most associated with incidental Lewy bodies at autopsy were slow "rapid alternating movements of hands", slow "hand movements", "rigidity", "body bradykinesia and hypkinesia", "action or postural tremor of hands", and "tremor at rest"

REPORTABLE OUTCOMES

Abstracts and scientific presentations :

- White LR, Ross GW, Petrovitch H, Morens DM, Grandinetti A. Mid-life Coffee Consumption Is Inversely and Independently Associated with the Subsequent Diagnosis of Parkinson's Disease in Japanese-American Men. Neurology 1999;52(Suppl 2):A539.
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- Ross GW, Tanner CM, Abbott RD, Petrovitch H, Davis DG, Nelson J, Markesbery W, Hardman J, White LR. Association of olfactory dysfunction with presence of Lewy bodies in the substantia nigra or locus ceruleus at autopsy: a prospective cohort study. Neurology 2000; 54 (Suppl 3):A391.
- Ross GW, Sharp DS, O'Callaghan JP, Petrovitch H, Miller DB, Nelson J, Launer LJ, White LR. Quantification of glial fibrillary acidic protein levels in neocortical regions of elderly Japanese American men with Alzheimer's disease. Neurobiology of Aging 2000; 21 (Suppl 1):S193.
- Ross GW, Abbott RD, Petrovitch H, Masaki KH, Tanner CM, Curb JD, Blanchette PL, White L. Mid-life Body Composition and the Future Risk of Parkinson's Disease. Presented at the 5th International Conference on Progress in Alzheimer's and Parkinson's Disease, Kyoto, Japan, April 2001.
- Petrovitch H, Ross GW, Abbott RD, Sanderson WT, Sharp DS, Tanner CM, Masaki KH, Blanchette PL, Popper JS, Foley D, White LR. Plantation Work and Risk of Parkinson's Disease in a Population-Based Longitudinal Study. Presented at the 5th International Conference on Progress in Alzheimer's and Parkinson's Disease, Kyoto, Japan, April 2001.
- White LR, Petrovitch H, Abbott RD, Masaki KH,Yano K, Grandinetti A, Popper JS, Ross GW Prolonged QT interval in midlife is a risk factor for subsequent development of Parkinson's disease. Presented at the 5th International Conference on Progress in Alzheimer's and Parkinson's Disease, Kyoto, Japan, April 2001.
- Abbott RD, Petrovitch H, White LR, Masaki KH, Tanner CM, Curb JD, Grandinetti A, Blanchette PL, Popper JS, Ross GW. Frequency of Bowel Movements and the Future Risk of Parkinson's Disease. Presented at the 5th International Conference on Progress in Alzheimer's and Parkinson's Disease, Kyoto, Japan, April 2001.
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- Ross GW, Petrovitch H, Nelson J, White LR, Davis D, Markesbery W, Hardman J, Fong K, Launer L. The relationship of Lewy bodies to Alzheimer lesions and cognitive function in a population based autopsy series. Neurobiology of Aging 2002; 23, (No. 1S) S419-S420. (Appendix L)

Manuscripts

- Ross GW, Abbott RD, Petrovitch H, Morens DM, Grandinetti A, Tung KH, Tanner CM, Masaki K, Blanchette PL, Curb JD, Popper JS, White LR. The association of coffee and caffeine intake with the risk of Parkinson's disease. JAMA 2000; 283:2674-2679. (Appendix

A)

- Abbott RD, Petrovitch H, White LR, Masaki KH, Tanner CM, Curb JD, Grandinetti A, Blanchette PL, Popper JS, Ross GW. Frequency of Bowel Movements and the Furture Risk of Parkinson's Disease. *Neurology* 2001;57(3):456-462. (Appendix E)
- Ross GW, Petrovitch H. Current evidence for neuroprotective effects of nicotine and caffeine against Parkinson's disease. Drugs and Aging 2001; 18(11): 797-806. (Appendix Q)
- Ross GW, Bowen, JD. The diagnosis and differential diagnosis of dementia. Med Clin North Am. 2002 May;86(3):455-76. (Appendix R)
- Abbott RD, Ross GW, White LR, Nelson JS, Masaki KH, Tanner CM, Curb JD, Blanchette PL, Popper JS, Petrovitch H. Mid-Life Adiposity and the Future Risk of Parkinson's Disease. Neurology 2002; 59:1051-1057. (Appendix G)
- Petrovitch H, Ross GW, Abbott RD, Sanderson WT, Sharp DS, Tanner CM, Masaki KH, Blanchette PL, Popper JS, Foley DJ, Launer LJ, White LR. Plantation Work and Risk of Parkinson's Disease in a Population-Based Longitudinal Study. Arch Neurol [In Press]. (Appendix D)
- Ross GW, O'Callaghan JP, Sharp DS, Petrovitch H, Miller DB, Abbott RD, Nelson JS, Launer LJ, Foley DJ, Burchfiel CM, White LR. Quantification of Regional Glial Fibrillary Acidic Protein Levels in Alzheimer's Disease. Acta Neurologica Scandinavica. [In Press] (Appendix N).
- White LR, Petrovitch H, Hardman J. Nelson J, Davis D, Ross GW, Masaki KH, Launer L, Markesbery W. Cerebrovascular pathology and dementia in autopsied Honolulu-Asia Aging Study participants. Ann N.Y. Acad. Sci [In Press]. (Appendix K)
- Abbott RD, Ross GW, White LR, Sanderson W, Burchfiel C, Kashon M, Sharp D, Masaki KH, Curb JD, Petrovitch H. Environmental, lifestyle, and physical precursors of clnical Parkinson's disease: recent findings from the Honolulu-Asia Aging Study.[In press]. (Appendix C)

Letter to the Editor

Ross GW, Abbott RD, Petrovitch H, White, LR. Relationship between caffeine intake and parkinson disease. [Letter] JAMA. 2000 Sep 20;284(11):1378-9. (Appendix S)

Additional funding received

- Department of Veterans Affairs Merit Review awarded 10/1/99 – 9/30/02 ($270,000) to Dr. Ross entitled The association of Parkinson's disease and parkinsonism in World War II veterans and other men. This is a three year project to re-examine the HHP/HAAS cohort for new cases of Parkinson's disease and parkinsonism related to other neurodegenerative and vascular causes. This project complemented this DOD funded study by adding additional cases of Parkinson's disease and parkinsonism for risk factor analyses.
- National Institute of Neurological Disorders and Stroke grant entitled "Neuropath markers of Parkinson's disease" was awarded to Dr. Ross.(9/15/2000-8/31/2004; $1,800,000) The aims of this four year project are to quantify two neuropathologic markers of PD: neuronal loss in the substantia nigra and diminished striatal dopamine levels in the brains of 600 deceased HAAS participants. These markers will be used as continuous endpoint variables to identify occupational, dietary and other environmental exposures that influence the development of these neuropathologic processes. This project complemented this DOD study by providing

additional neuropathological outcomes for neurotoxic exposure analysis

- A supplement to the project entitled Risk Factors for Pathologic Markers of Parkinson disease was funded by the National Institute of Neurological Diseases and Stroke to examine the association of simple and choice reaction time with striatal levels of dopamine and neuron counts in the substantia nigra ($49,053 for one year).
- National Institute on Aging grant entitled "Epidemiology of brain aging in the very old" was awarded to Dr. Lon White. (Dr. Ross is Co-PI). (4/01-3/06; 7,720,716) A five year study to re-examine the Honolulu-Asia Aging Study cohort for incident dementia and Parkinson's disease.
- National Institute of Neurological Disorders and Stroke grant entitled "Parkinson's Disease neuroprotection trial: Hawaii center" awarded to Dr. Ross. (9/30/02-8/30/07; $536,768) This is a randomized, double blind, placebo controlled clinical trial to determine the efficacy of two or more agents as neuroprotective therapy for early Parkinson's disease.
- National Institute for Environmental Health Sciences grant entitled "Environmental, Genetic and Cellular Determinates of Parkinson's disease" awarded to the Parkinson's Institute in Sunnyvale, CA (Dr. William Langston, PI) This award was given in response to an RFA from NIEHS for Collaborative Centers for Parkinson's disease environmental research. Dr. Ross is collaborating with Dr. Caroline Tanner from the Parkinson's Institute on a multi-center project to evaluate genetic andenvironmental influences on Parkinson's disease. (8/1/02-7/31/07; Dr. Ross is awarded $107,000 through a subcontract to Pacific Health Research Institute.)

Other research opportunities .

- Honolulu parkinsonism study investigators have established a collaboration with Dr. Caroline Tanner, a Movement Disorders specialist and epidemiologist from the Parkinson's Institute in Sunnyvale, California. Dr. Tanner has provided excellent advice on clinical diagnosis of parkinsonism, as well as assistance with data analysis and manuscript preparation. This collaboration led to Dr. Ross being invited to spend a sabbatical year at the Parkinson's Institute 7/00-6/01.
- Honolulu parkinsonism study investigators have established a collaboration with Jim O'Callaghan, PhD, Diane Miller, PhD, and Dan Sharp, MD, PhD, scientists from the National Institute of Occupational Safety and Health to look for markers of brain injury. A pilot project of glial fibrillary acidic protein in the brains of Alzheimer's disease has already been completed. Additionally, Dr. Miller's lab is performing dopamine and dopamine metabolite levels on caudate and putamen frozen specimens as part of Dr. Ross' NINDS project entitled Risk Factors for Pathologic Markers of Parkinson disease (see above).
- Collaboration with Dr. Maryka Quik from the Parkinson's Institute in Sunnyvale, CA. The goal of this work is to identify the nicotinic receptor subtypes in human caudate-putamen and determine the changes that occur in Parkinson's disease in never and ever smokers. As a first step we have sent frozen caudate and putamen to Dr. Quik's lab from the brains of 15 heavy smokers and 18 never smokers to assess the effects of smoking on nicotinic receptor subtypes. Later, we will compare nicotinic receptor subtypes in caudate and putamen from smoking and nonsmoking subjects who had Parkinson's disease as well as controls. It is hoped that this knowledge could be used for developing new therapies for Parkinson's disease (see Appendix Q).
- 19th International Neurotoxicology Conference : The title of this years conference held August 25-28, 2001 in Colorado Springs, CO was "Parkinson's disease, Environment, and Genes.

The P.I. was invited to give a talk entitled "Environmental factors inversely associated with Parkinson's disease."

- National Institute of Environmental Health Sciences Parkinson's disease epidemiology workshop: The P.I. was invited to participate in a second workshop on the epidemiology of Parkinson's disease sponsored by the National Institute of Environmental Health Sciences held in Sunnyvale, CA 2001. The purpose of this two day workshop was to discuss data on environmental risk factors being collected in epidemiologic studies of PD, methods to assess exposures, data on genetic markers, and opportunities for collaborative analyses such as data pooling, meta-analysis, or parallel analysis. A manuscript is in preparation that will be a pooled analysis of smoking and PD from data submitted by nine centers that were participants in the workshop. It is hoped that by pooling these data there will be enough statistical power to examine unresolved questions regarding the relationship between smoking and PD. These include the effects of starting and stopping smoking as well as the amount and total duration of smoking on the relationship.
- Behavioral Toxicology Society annual meeting held May 5, 2001 in Raleigh-Durham, NC: The P.I. was invited to speak at a symposium entitled "Neurological Diseases and Neurotoxicology" on the epidemiology of Parkinson's disease and the link with pesticide exposure.
- Environmental Factors in Parkinson's Disease Workshop: The P.I. was invited to participate in this workshop sponsored by The Michael J. Fox Foundation and held at the Parkinson's Institute in Sunnyvale, CA December 13, 2001. The purpose of this workshop was to identify specific ways the Foundation could further the study of environmental factors that contribute to the development and progression of Parkinson's disease.

CONCLUSIONS

The work accomplished with our DOD funding has led to numerous opportunities for dissemination of our research findings at scientific meetings, symposia, and workshops over the four years of the project. Valuable collaborations have been developed with the Parkinson's Institute and the National Institute for Occupational Safety and Health (NIOSH)

The findings that coffee drinking and caffeine consumption is associated with a lower risk of Parkinson's disease and a lower risk of incidental Lewy bodies suggest that caffeine may play a role in therapy for or prevention of PD. Importantly, these findings have been replicated in another prospective study[29] and studies using MPTP models of parkinsonism suggest that caffeine and caffeine-like compounds may be neuroprotective[30].

Our findings, determined prospectively, of associations of years worked on a plantation, exposure to pesticides, and occupation in a sugar refinery with incident Parkinson's disease have corroborated case control reports suggesting as association of environmental neurotoxins with Parkinson's disease. Frozen samples from two brain regions of 15 deceased HHP/HAAS participants with highest exposure to organochlorines during life have been analyzed and results indicate that organochlorines are detected in most of these brains that were exposed as long as 30 years ago. These encouraging findings have led to a request for supplemental funding to assay all the brains in the HAAS autopsy series for organochlorines. We will examine the association of these levels with clinical endpoints (Parkinson's disease, parkinsonism, Alzheimer's disease, cognitive impairment) and pathological endpoints (lewy bodies, neuritic plaques, neurofibrillary tangles, cell counts in the substantia nigra, and striatal dopamine levels). Our ability to measure levels of organochlorine compounds in the brains of deceased participants is important for several

reasons. Such data could provide direct evidence linking specific neurotoxin exposures to neurodegenerative conditions, prominently including Parkinson's disease. Although many epidemiological studies have implicated insecticides through self report, few studies have been performed that directly measure specific organochlorines in brain and report an association between these levels and PD.

Analyses demonstrating that increased triceps skinfold thickness, a measure of peripheral adiposity, may precede the extrapyramidal syndrome by years suggest that metabolic differences in those at higher risk for developing Parkinson's disease may be present years before the motor syndrome develops. We have also reported that longer QT interval on ECG and constipation during mid-life may portend the onset of the motor syndrome of PD by years. Additionally, the finding that slow reaction time is a predictor for incidental Lewy bodies suggests that this test may be useful in the early detection of Parkinson's disease. These findings along with the association of impaired olfaction with incidental Lewy bodies suggest that the identification of such early markers could be used to identify individuals at high risk for the development of PD. Persons so identified would be candidates to participate in drug studies aimed at disease prevention and/or might be preferentially excluded from subsequent exposure to agricultural or military chemicals having possible neurotoxicity.

The genotyping for several genetic polymorphisms possibly associated with PD has been completed for 117 cases and 240 controls. Results have been negative in preliminary statistical analyses.

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Ref Type: Journal (Full)

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APPENDIX A

ORIGINAL CONTRIBUTION

Association of Coffee and Caffeine Intake With the Risk of Parkinson Disease

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Context The projected expansion in the next several decades of the elderly population at highest risk for Parkinson disease (PD) makes identification of factors that promote or prevent the disease an important goal.

Objective To explore the association of coffee and dietary caffeine intake with risk of PD.

Design, Setting, and Participants Data were analyzed from 30 years of follow-up of 8004 Japanese-American men (aged 45-68 years) enrolled in the prospective longitudinal Honolulu Heart Program between 1965 and 1968.

Main Outcome Measure Incident PD, by amount of coffee intake (measured at study enrollment and 6-year follow-up) and by total dietary caffeine intake (measured at enrollment).

Results During follow-up, 102 men were identified as having PD. Age-adjusted incidence of PD declined consistently with increased amounts of coffee intake, from 10.4 per 10000 person-years in men who drank no coffee to 1.9 per 10000 person-years in men who drank at least 28 oz/d ($P<.001$ for trend). Similar relationships were observed with total caffeine intake ($P<.001$ for trend) and caffeine from noncoffee sources ($P=.03$ for trend). Consumption of increasing amounts of coffee was also associated with lower risk of PD in men who were never, past, and current smokers at baseline ($P=.049$, $P=.22$, and $P=.02$, respectively, for trend). Other nutrients in coffee, including niacin, were unrelated to PD incidence. The relationship between caffeine and PD was unaltered by intake of milk and sugar.

Conclusions Our findings indicate that higher coffee and caffeine intake is associated with a significantly lower incidence of PD. This effect appears to be independent of smoking. The data suggest that the mechanism is related to caffeine intake and not to other nutrients contained in coffee.

JAMA. 2000;283:2674-2679 www.jama.com

PARKINSON DISEASE (PD) AFflicts 3% of the population older than 65 years[1] and is a significant source of morbidity and health services use. Based on the projected growth of the US population, this percentage could double in the next 30 to 40 years.[2] While rare genetic forms exist, determinants of typical late-onset disease appear to be largely environmental.[3,4] No treatment has definitively been shown to prevent disease or slow progression. Identification of risk factors may lead to an understanding of pathogenic mechanisms and to effective strategies for prevention.

Coffee intake has been inversely associated with PD occurrence in some studies, but evidence has been equivocal.[5-8] In an earlier longitudinal study from the Honolulu Heart Program, coffee intake measured prospectively appeared to be protective against PD, but not after adjustment for cigarette smoking.[5]

This article presents an expanded analysis of the relationship between consumption of coffee and dietary caffeine and risk of PD within the Honolulu Heart Program cohort, based on longer follow-up and nearly twice the number of incident PD cases than were previously available.[5] The role of other nutrients contained in coffee are also examined.

METHODS

The Honolulu Heart Program was established in 1965 with the examination of 8006 men of Japanese ancestry 45 to 68 years old and living on the island of Oahu, Hawaii. The initial examination consisted of face-to-face

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interviews and physical evaluation. Demographic, dietary, and health status data were obtained.[9,10] The study is now in its 34th year of follow-up with continued surveillance of hospitalization and death records. Follow-up examinations were performed from 1968 to 1971, 1971 to 1974, 1991 to 1993, and 1994 to 1996. Research on neurodegenerative diseases of aging began in 1991 with establishment of the Honolulu-Asia Aging Study. Procedures were approved by an institutional review committee and informed consent was obtained from all participants. Details regarding study design have been previously published.[5,11,12]

PD Case Finding and Diagnosis

For this report, 30 years of follow-up data were available. Incident cases of PD were identified through 4 sources.[13] Prior to 1991, the sources were: (1) review of all cohort members' hospitalization records for all diagnoses of PD, (2) ongoing review of all Hawaii death certificates, and (3) review of medical records of all patients with PD from the offices of local neurologists cross-checked with the cohort member list.[5,13]

After 1991, the diagnosis of PD was based on complete reexaminations of the entire cohort from 1991 to 1993 and 1994 to 1996. During the 1991 to 1993 examination,[13] all subjects were questioned about history of PD, symptoms of parkinsonism (tremor, bradykinesia, rigidity, or postural instability), and PD medications by structured interview. Research technicians were trained to recognize clinical signs of parkinsonism including gait disturbance, tremor, or bradykinesia. Subjects with a history of PD or parkinsonism symptoms or signs were referred to a study neurologist who administered standardized questions about symptoms and onset of parkinsonism, previous diagnoses, and medication usage, followed by a comprehensive and standardized neurological examination that included the Unified Parkinson's Disease Rating Scale.[14] Diagnosis of PD was based on consensus from 2 neurologists according to published criteria.[15] These require that the subject have the following: (1) parkinsonism; (2) a progressive disorder; (3) any 2 of marked response to levodopa, asymmetry of signs, asymmetry at onset, or initial onset tremor; and (4) absence of any etiology known to cause similar features. Cases of parkinsonism related to other neurodegenerative disorders, cerebrovascular disease, medications, trauma, or postencephalitic parkinsonism were not included among cases of PD. Additional cases of PD were identified during the 1994 to 1996 examination through structured interviews inquiring about history of PD or PD medications. These cases were confirmed by a study neurologist through record review and application of the criteria above.

Age at diagnosis was used instead of age at onset to avoid inaccuracies associated with recall of symptom onset for a chronic disease with gradual onset. At study enrollment, there were 2 prevalent cases of PD excluded from this analysis, leaving 8004 available for prospective follow-up.

Measurement of Coffee Intake and Other Covariates

At study enrollment (1965-1968), nutrient intake was determined by a dietitian based on 24-hour dietary recall methods.[16] The 24-hour dietary recall was validated against a full week dietary record for 329 of the 8006 men in the original cohort. Comparison between the 2 assessments showed no significant differences in mean intake of 9 nutrients.[16] Coffee was assessed as caffeinated only (decaffeinated coffee was not assessed) and intake was measured as the number of 4-oz cups in the 24 hours encompassed by the intake record. (To convert ounces to milliliters, multiply by 30.) Intake categories were then defined as none, 4 to 8 oz/d, 12 to 16 oz/d, 20 to 24 oz/d, and 28 oz/d or more. Dietary recall also assessed intake of milk and sugar (separately and as additives to coffee), as well as green tea, black tea, other caffeinated beverages, and caffeine from other sources. Six years later (1971-1974), as part of a food frequency questionnaire, subjects were asked about coffee intake in the prior week and if the average serving size was small (4 oz), medium (6 oz), or large (8 oz). For this examination, total coffee intake was assessed without regard to caffeinated vs decaffeinated. Intake was converted to average daily consumption defined as none, more than 0 to 8 or less oz/d, more than 8 to 16 or less oz/d, more than 16 to 24 or less oz/d, and more than 24 oz/d.

Total dietary caffeine and dietary caffeine from noncoffee sources were calculated from the baseline 24-hour dietary intake record. Most caffeine from noncoffee sources came from tea or cola beverages, with a small proportion from chocolate. Subjects were classified by quintiles of caffeine intake per day for both measurements. Other nutrients were determined from the same 24-hour dietary recall based on consumption of individual food items using computer software (Nutritionist IV, N-Square Computing, Salem, Ore). Multiple nutrients were examined, including niacin. Data on dietary caffeine and other nutrients were available only from the baseline 1965 to 1968 examination. Pack-years of smoking were assessed at study enrollment (1965-1968) and 6 years later (1971 to 1974). Other dietary measures from the baseline examination included total energy intake and saturated fat level. Alcohol consumption, total serum cholesterol level, and physical activity were also assessed at enrollment.

Statistical Methods

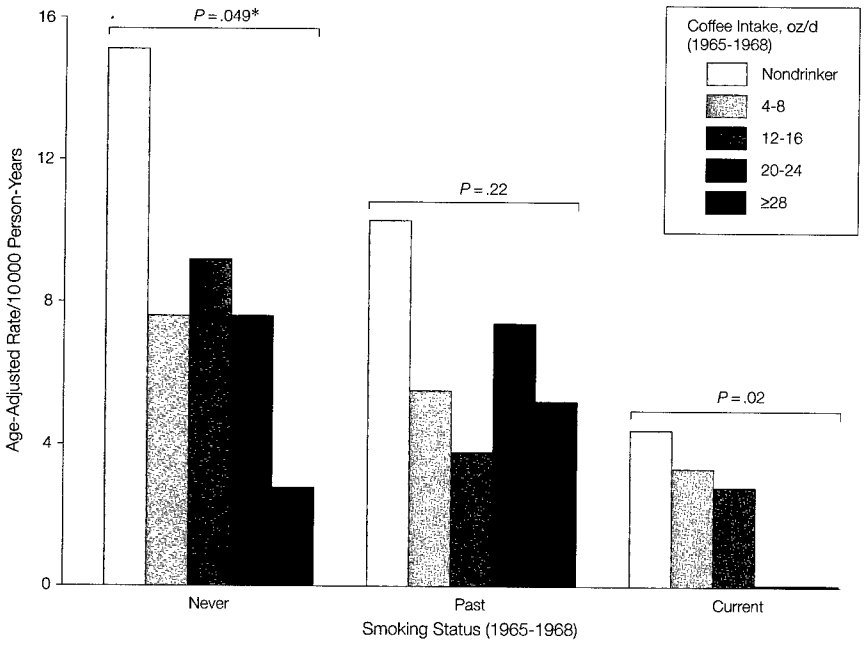
Incidence rates in person-years were estimated within categories of coffee consumption based on 30 years of follow-up for the 8004 men whose intake was determined at the 1965 to 1968 baseline examination. Similar rates were derived according to quintiles of total caffeine intake and for caffeine intake from noncoffee sources. Incidence rates were similarly estimated according to categories of coffee intake based on 24 years of follow-up for the 5933 men whose intake was also determined 6 years later (1971-1974). Unadjusted and age-adjusted incidence rates are provided.[17]

Table 1. Unadjusted and Age-Adjusted Incidence of Parkinson Disease (PD) According to Amounts of Coffee Consumed per Day

Coffee Intake, oz/d	No. of Cases of PD/No. of Subjects at Risk	Incidence Rate/10 000 Person-Years		Adjusted Relative Hazard (95% CI) Compared With the Top Category of Coffee Intake*
		Unadjusted	Adjusted for Age	
Based on 30 Years of Follow-up After the 1965 to 1968 Examinations				
Nondrinker	32/1286	10.5	10.4	5.1 (1.8-14.4)†
4 to 8	33/2576	5.5‡	5.3§	2.7 (1.0-7.8)
12 to 16	24/2149	4.7‡	4.7‡	2.5 (0.9-7.3)
20 to 24	9/1034	3.6‡	3.7‡	2.0 (0.6-6.4)
≥28	4/959	1.7‖	1.9‖	Reference
Test for trend	. . .	$P<.001$	$P<.001$	$P<.001$
Nondrinkers vs drinkers	. . .	. . .	. . .	2.2 (1.4-3.3)¶
Based on 24 Years of Follow-up After the 1971 to 1974 Examinations				
Nondrinker	17/539	17.4	17.3	3.0 (1.1-8.4)#
>0 to ≤8	25/2383	5.6‖	5.4‖	1.1 (0.4-2.9)
>8 to ≤16	16/1445	5.9‡	5.9‡	1.1 (0.4-3.0)
>16 to ≤24	8/989	4.2‖	4.3‖	0.8 (0.3-2.6)
>24	5/577	4.6‡	5.0‡	Reference
Test for trend	. . .	$P = .008$	$P = .005$	$P = .03$
Nondrinkers vs drinkers	. . .	. . .	. . .	2.9 (1.7-5.1)¶

*Adjusted for age and pack-years of cigarette smoking. CI indicates confidence interval.
†Significant excess risk of PD, $P<.01$.
‡Significantly different from nondrinkers, $P<.01$.
§Significantly different from nondrinkers, $P<.05$.
‖Significantly different from nondrinkers, $P<.001$.
¶Significant excess risk of PD, $P<.001$.
#Significant excess risk of PD, $P<.05$.

Figure 1. Age-Adjusted Incidence of Parkinson Disease



Age-adjusted incidence based on 30 years of follow-up according to coffee intake at the time of study enrollment (1965-1968) for those who were never, past, and current cigarette smokers. Asterisk indicates test for trend.

To test the possibility that the effect of coffee on PD changed over time and to estimate the independent effect of coffee and caffeine intake on risk of PD after adjusting for age and pack-years of cigarette smoking, proportional hazards regression models were used.[18] In addition, coffee and caffeine intake were modeled as continuous variables. The significance of the regression coefficients that were associated with coffee and caffeine when modeled as continuous variables comprised a test for trend or a test for a dose-response relationship between coffee intake and risk of PD. Relative hazards of PD (and associated confidence intervals) were estimated comparing risk of disease between amounts of coffee consumed. All reported P values were based on 2-sided tests of significance. Alcohol was modeled as a continuous measure in the number of grams per day consumed. Other covariates were also modeled as continuous variables (saturated fat level, physical activity, total energy intake, and total serum cholesterol level). Hypertension and diabetes were modeled through the use of indicator variables.

RESULTS

The median age of the 8004 men at study enrollment (1965-1968) was 53 years (range, 45-68 years). The median length of follow-up was 27 years, minimum follow-up was 0.8 years to the first death, and maximum follow-up was 30 years from the baseline examination. Among the men, 102 developed PD over the 30 years of follow-up. The median age of PD diagnosis was 73.6 years (range, 54-89 years), and the median interval between baseline examination and PD onset was 16.6 years (range, 2-30 years).

Coffee drinkers had significantly lower incidence of PD than nondrinkers ($P<.001$). This effect was apparent when examining incidence of PD based on 30 and 24 years of follow-up according to amounts of coffee consumed at the time of study enrollment and at the 1971 examination (TABLE 1). At each examination, increasing

amounts of coffee consumed were associated with decline in PD incidence ($P<.01$). For nondrinkers of coffee, after adjustment for age and pack-years of cigarette smoking, risk of PD was 2 to 3 times greater than for reported coffee drinkers ($P<.001$). Based on data collected at the time of study enrollment, nondrinkers of coffee had a risk of PD more than 5 times that of men who consumed 28 oz of coffee or more per day ($P<.01$).

The progressively lower risk of PD with increasing amounts of coffee consumed was also observed in men who were never, past, and current smokers (FIGURE 1). For consumption determined at the baseline examination, the dose trend was statistically significant for men who never smoked cigarettes ($P=.049$) and for current smokers ($P=.02$).

The incidence of PD by quintile of caffeine intake at study enrollment (1965-1968) was examined for both total caffeine and caffeine from sources other than coffee (TABLE 2). For both sources of caffeine, dose relationships with PD development were similar to those shown for coffee consumption.

Although the protective effect of dietary caffeine showed a similar dose-response pattern for both drinkers and nondrinkers of coffee, it was significant only in coffee drinkers. The lack of a significant association in noncoffee drinkers may have been due to a small sample size.

Cumulative incidence curves for PD over time by amounts of coffee consumed and by caffeine intake from noncoffee sources reveal the magnitude of dose effect between exposure categories (FIGURE 2). For men who were nondrinkers of coffee and those who consumed 28 oz or more per day, differences in the cumulative incidence of PD became apparent as early as 10 years into follow-up (Figure 2, top). A similar divergence is apparent between men who consumed the least and the most amounts of caffeine from noncoffee sources (Figure 2, bottom).

As noted earlier, methods for case finding changed after 1991. This had no effect on the observed relationships between PD and coffee or caffeine intake. The association between coffee intake at study enrollment and risk of PD remained statistically significant for men whose diagnosis of PD occurred before ($P=.005$) and after 1991 ($P=.01$).

Coffee intake determined at study enrollment was also significantly associated with PD that occurred in the first ($P=.048$) and second ($P=.002$) 15 years of follow-up. In both instances, risk of PD declined with increasing amounts of coffee consumed.

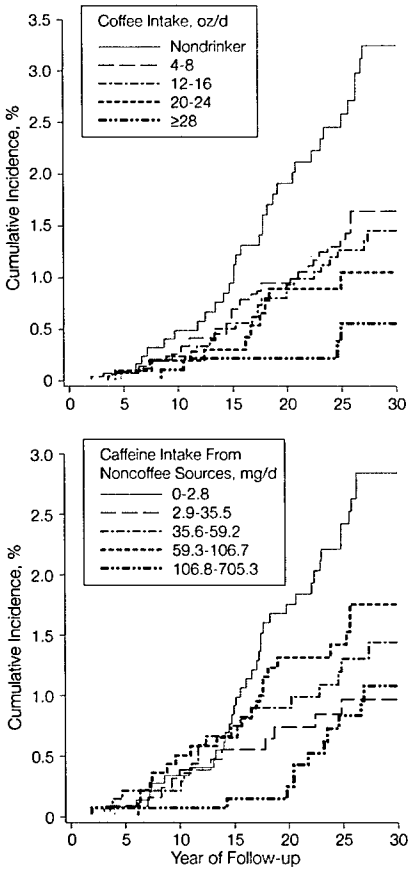
Among the other nutrients contained in coffee that were analyzed, including niacin, no associations were observed with risk of PD nor did they alter the association between coffee and caffeine intake with risk of PD. Adjustment for alcohol consumption, hyper-

Table 2. Unadjusted and Age-Adjusted Incidence of Parkinson Disease (PD) According to Total and Noncoffee Amounts of Caffeine Consumed per Day Based on 30 Years of Follow-up Beginning From 1965 to 1968

Quintile of Caffeine Intake, mg/d	No. of Cases of PD/No. of Subjects at Risk	Incidence Rate/10 000 Person-Years		Adjusted Relative Hazard (95% CI) Compared With the Top Category of Caffeine Intake*
		Unadjusted	Adjusted for Age	
Total Caffeine				
0-123	35/1522	9.8	9.7	5.1 (2.1-12.3)†
124-208	17/1396	5.2‡	5.0‡	2.6 (1.0-6.6)
209-287	26/1607	6.8	6.7	3.8 (1.6-9.3)§
288-420	12/1485	3.4‖	3.4‖	2.0 (0.7-5.3)
421-2716	6/1481	1.7¶	1.8¶	Reference
Test for trend	...	$P<.001$	$P<.001$	$P<.001$
Caffeine From Noncoffee Sources				
0-2.8	35/1642	9.2	9.2	2.7 (1.4-5.4)§
2.9-35.5	11/1389	3.4‖	3.4‖	1.2 (0.5-2.7)
35.6-59.2	18/1486	5.1‡	5.1‡	1.6 (0.7-3.3)
59.3-106.7	21/1487	5.9	5.9	1.9 (0.9-4.0)
106.8-705.3	11/1487	3.1‖	3.1‖	Reference
Test for trend	...	$P = .03$	$P = .03$	$P = .03$

*Adjusted for age and pack-years of cigarette smoking. CI indicates confidence interval.
†Significant excess risk of PD, $P<.001$.
‡Significantly different from nondrinkers, $P<.05$.
§Significant excess risk of PD, $P<.01$.
‖Significantly different from nondrinkers, $P<.01$.
¶Significantly different from nondrinkers, $P<.001$.

Figure 2. Cumulative Incidence of Parkinson Disease



Cumulative incidence according to coffee intake (top panel) and caffeine intake from noncoffee sources (bottom panel) at the time of study enrollment (1965-1968).

tension, cholesterol level, total energy intake, and saturated fat level had no effect on results of the model. Consumption of milk and sugar also failed to alter the reported findings.

COMMENT

To our knowledge, this is the first prospective study demonstrating a significant inverse association between coffee consumption measured during midlife and incident PD with a dose-response relationship. The finding was consistent whether coffee intake was determined by 24-hour recall or by food frequency questionnaire. The association was also observed for coffee intake measured at different examinations 6 years apart. Based on estimates of total or noncoffee caffeine and other nutrients contained in coffee derived from information collected at study inception, it appears caffeine may be the responsible constituent.

Previous studies have also suggested coffee consumption may be inversely related to risk of PD. In an earlier article from the Honolulu Heart Program, based on 58 cases also included in the case panel presented here, Grandinetti and colleagues[5] reported that coffee drinking was inversely related to PD, although the association was not statistically significant after controlling for cigarette smoking and alcohol consumption. The current report, based on longer follow-up and additional PD cases, found coffee drinking to be inversely related to PD risk independent of smoking and alcohol. Although 2 retrospective studies found that persons with PD were less likely to be coffee drinkers than persons without PD, the results were not statistically significant.[7,8] In 2 other case-control studies, individuals with PD consumed significantly less coffee prior to the diagnosis of PD than controls.[6,19] In both studies, a significant inverse dose-response relationship between coffee intake and PD was observed. However, the authors noted that retrospective assessment of coffee intake could be biased by current dietary habits.[6]

The lower frequency of coffee consumption during midlife among men who eventually developed PD could reflect a psychological or physiological intolerance to caffeine among persons with a constitutional propensity to develop PD. Alternatively, regular exposure to caffeine over many years might counteract the aging-related neurodegenerative processes that cause loss of dopaminergic neurons.

The pharmacological effects of caffeine could also modulate neurotransmitter and receptor systems of brainstem pigmented nuclei or striatum. Caffeine is a known central nervous system stimulant thought to act through adenosine receptor antagonism. Adenosine receptor agonists produce decreased locomotor activity in rodents, possibly through inhibition of dopamine neurotransmission.[20,21] Recent reports indicate that adenosine A2 receptor antagonists improve motor deficits in primates treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).[22,23] Caffeine given to mice with pharmacologically induced dopamine depletion prevents akinesia.[20] This dopaminelike effect may be related to removal of tonic inhibition by adenosine on dopaminergic neurotransmission rather than direct stimulation of dopamine receptors by caffeine.[20,24] Thus, rather than having a direct biological effect on the pathogenesis of PD, coffee and other caffeine sources may be a form of self-medication that decreases clinical expression of parkinsonism by increasing central dopaminergic tone. Clinical studies do not consistently support this, however. Two small clinical trials of caffeine given concomitantly with either a dopamine agonist or levodopa to patients with PD have demonstrated no increased efficacy with caffeine.[25,26] One open-label trial of theophylline, another adenosine receptor antagonist, in 15 parkinsonian patients appeared to show improvement in disability scores.[27]

Two case-control studies[6,19] indicated that niacin contained in coffee might be neuroprotective. However, micronutrient analysis in this study included niacin, and this hypothesis was not supported.

Other explanations for our findings must be considered. If coffee consumption were associated with increased mortality, then selective survival of noncoffee drinkers may explain the inverse relationship between coffee and PD. A previous article from the Honolulu Heart Program found that coffee drinking is associated with higher cholesterol levels.[28] If this effect were enough to increase cardiovascular mortality, then heavy coffee drinkers may have been more likely to die before developing PD. This is not likely in our analysis. Adjustment for cholesterol level had no effect on the results and no association was found between coffee drinking and mortality ($P=.90$). Finally, an earlier article from the Honolulu Heart Program shows no relationship between coffee drinking and coronary artery disease risk.[29]

Incomplete PD case ascertainment among heavy coffee drinkers could also lead to an apparent protective effect of coffee drinking if heavy coffee intake were associated with not participating in follow-up examinations. To evaluate the possibility of missed cases in the heavy coffee-consuming group, additional analyses were performed to examine participation in the 1971 and 1991 examinations based on coffee consumption at the 1965 examination. There was no trend for nonparticipation in subsequent examinations with increased coffee consumption at the baseline examination. Similarly, there was no trend for nonparticipation in the 1991 examination based on coffee consumption at the 1971 examination. Since coffee drinking is not associated with mortality or with nonparticipation at subsequent examinations, it is unlikely that missed cases due to nonparticipation were preferentially heavy coffee drinkers. Associations between coffee and PD were also similar and statistically significant between the first and second 15 years of follow-up.

One other possibility is that individuals destined to develop PD used caffeine-containing analgesics and other medications more commonly than others and reduced their coffee intake to

avoid excess caffeine. Because consumption of nondietary caffeine-containing products was not assessed in the Honolulu Heart Program, this issue cannot be addressed.

There are potential limitations to this study. The population is Japanese-American men with older age at diagnosis. A recent report of concordance of PD among twins[3] suggests that older-onset PD may be more likely related to environmental factors compared with younger-onset cases with a stronger genetic component. This implies that when assessing environmental risk factors for PD, the use of an older population may improve chances of a successful yield.

Generalizations to younger-onset cases, women, and other ethnic groups cannot be made with certainty. A recent review of the worldwide frequency of PD suggests that incidence is somewhat lower in Japan, with crude incidence rates ranging from 5.4 to 10.2 compared with rates in northern Europe (range, 6-16), and Rochester, Minn (crude incidence, 19.7-23).[30] Those studies with the highest rates included all forms of parkinsonism (including drug induced and vascular) as cases. These data have been interpreted to suggest that PD may be more common in whites; however, it is entirely possible that differences are related to case finding and case definition methods.[31] Although the cause of PD is not known, the clinical syndrome and neuropathological characteristics are identical and risk factor profiles are similar in ethnic groups worldwide.[31]

Most important, the observational nature of the study design prevents concluding that coffee or caffeine directly protect against development of PD. However, prospective assessment of exposures and unbiased case-finding methods are unique strengths that enhance the importance of the findings. The possibility that caffeine has a protective effect against PD should be investigated further with future epidemiological, clinical, and basic science research.

Funding/Support: This work was supported by United States Department of the Army grant DAMD17-98-1-8621; National Institutes of Health, National Institute on Aging contract NO1-AG-4-2149; National Heart, Lung, and Blood Institute contract NO1-HC-05102; and VA Medical Research funds.

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Printed and Published in the United States of America

High Fruit intake, but not dietary vitamin C intake, in midlife predicts development of Parkinson's disease: The Honolulu-Asia Aging Study.

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Running head: Fruit intake and Parkinson's disease

Key words: Parkinson's disease, risk factor, epidemiology

Supported by a grant from the U. S. Department of the Army (DAMD17-98-8621), by the National Institures of Health (National Institute on Aging contract N01-AG-4-2149) and grant 1-R01-Ag17155-01A1, National Heart, Lung, and Blood Institute contract N01-HC-05102), by the National Institute of Occupational Safety and Health (Contract HELD0080060), and by the Office of Research and Development, Department of Veterans Affairs. The information contained in this article does not necessarily reflect the position or the policy of the Government, and no official endorsement should be inferred.

Abstract

Background: A recent report from the French West Indies linked tropical fruit consumption with atypical parkinsonism and supranuclear palsy. Several other studies have linked fruit and vitamin C intake to Parkinson's disease (PD) risk. However, these studies have been primarily retrospective in design, and are subject to recall bias. We examined the role of dietary factors as predictors of PD in a longitudinal study.

Methods: Risk factor data was obtained from a cohort of 8006 Japanese-American men aged 45-65 at a baseline examination in 1965, at three follow-up examinations, and via mail-out questionnaires. Incident PD cases were detected over 34 years of observation. Relative risk for developing PD was estimated by proportional hazard models, controlling for age, cigarette smoking, caffeine intake, past plantation work, and bowel frequency.

Findings: Increased fruit and fruit drink consumption predicted PD risk after adjusting for known PD risk factors. High dietary vitamin C intake, did not predict PD risk, after controlling for age, bowel frequency, smoking and coffee drinking. Likewise, use of vitamin C supplementation did not appear to be associated with PD risk.

Interpretation: These findings suggest that fruit consumption, rather than vitamin C intake, is associated with increased PD risk. We speculate that this increased risk may be due to plant borne toxins, or exogenous toxins such as pesticides or herbicides on PD risk. Further research to elucidate the role of food-borne toxins

may provide further insights into the etiology and prevention of Idiopathic Parkinson's disease.

Background

A study in the Caribbean reported a very strong association between tropical fruit consumption and progressive supranuclear palsy and atypical parkinsonism [1]. Several studies examining diet and dietary vitamin intake also reported increased fruit consumption or increased vitamin C intake among cases of Parkinson's disease [2-4]. Other studies compared concentrations of lipid soluble antioxidants such as alpha-tocopherol and vitamin A in serum and brain tissue [5-12]. The only study that observed a significant difference reported that vitamin C levels were higher among cases than controls [10]. Quantitative studies of dietary intake of antioxidant vitamins have also been performed, but these studies have reported conflicting results [3,4,13-17].

Caparros-Lefebvre suggested that high concentrations of neurotoxic benzyltetrahydroisoquinoline alkaloids may explain the association between tropical fruit consumption, PSP and atypical parkinsonism observed in their study [1]. An alternatively, vitamin C obtained from fruit consumption may have potential to act as either an antioxidant and prooxidant [18]. Cathcart proposed that vitamin C has much greater importance as a prooxioxidant than is usually recognized [19], while others caution that vitamin C may have neurotoxic effects [20].

All but one of the previous dietary studies [16] and all of the studies examining serum or tissue antioxidants assessed vitamin C after the onset of PD, therefore, a cause-and-effect bias may have arisen from alterations in dietary habits among the PD case patients. We report here findings from a longitudinal study among a

cohort consisting of 8,006 male Japanese-Americans enrolled in the Honolulu Heart Program, using dietary data collected at the initial baseline examination and subsequent follow-up examinations.

Methods

Study Sample

Data were obtained from the Honolulu Heart Program (HHP), an ongoing cohort study of 8,006 men of Japanese and Okinawan ancestry born between 1900 and 1919, and residing on Oahu, Hawaii in 1965. Details of the HHP methods have been described previously [21]. A total of 6,860 men, approximately 90% of surviving members, participated in the third examination. An institutional review committee approved procedures, and informed consent was obtained from all participants at each examination.

PD Case Ascertainment

A total of 141 cases with PD were identified between the baseline examination and 1999. Cases were identified through four sources: 1) review of Oahu hospitalization records; 2) review of Hawaii death certificates; 3) review of medical records at the offices of eight of ten Oahu neurologists; and 4) from a complete re-screening of the cohort (1991-1994). The re-screening included a neuropsychological screening and a detailed medical history that included standardized questions about parkinsonism diagnoses, medication usage, and signs and symptoms of parkinsonism. Suspected cases were given a comprehensive neurological examination by a study neurologist and final diagnoses were based on a consensus panel. Criteria for exclusion from analysis included parkinsonism related to progressive supranuclear palsy, multisystem

atrophy, cerebrovascular disease, as well as drug-induced parkinsonism, post-encephalitic parkinsonism, or post-traumatic parkinsonism.

Dietary Vitamin C Intake

Information gathered during the baseline HHP examination included a 24-hour dietary recall interview, and a food frequency of selected culturally significant foods [22] conducted at both the baseline examination (Exam 1) and at the third examination (Exam 3, 1971 to 1974). Dietary intakes of macro- and micronutrients were calculated for the 24-hour dietary recall using Nutritionist IV software (N-Squared Computing, San Bruno, CA), which uses the USDA database. The nutrient composition database was modified to accommodate foods and food preparation methods unique to the study population. Dietary vitamin C intakes were estimated from 7,625 complete 24-hour recall interviews.

Vitamin C supplement use was evaluated via a mail-out questionnaire completed and returned by 4,655 participants from 1989-1991. This comprehensive questionnaire included a food frequency questionnaire as well as questions on supplement use. Participants were asked for the frequency and potency of vitamin E, C, A, and multivitamin intake.

Statistical methods

Known PD risk factors were examined in relation to dietary vitamin C intake in order to identify factors that may introduce spurious associations between vitamin C intake and PD risk. Crude and age-adjusted incidence rates of PD in person-years were estimated for participants with high and low vitamin C intake at the baseline examination. Person-years were calculated separately for incident cases occurring after the third follow-up examinations and the mail-out dietary survey. The maximum time of follow-up available from 8,004 men who

participated in the baseline examination was 34 years. Proportional hazards regression models were used to test the null hypothesis that there was no difference in PD risk attributable to increased vitamin C, fruit and fruit juice intake. Multivariable models adjusted for known PD risk factors concurrently associated with vitamin C intake. Tests for trend for dose-response were performed by regressing PD risk on continuous variables, such as vitamin C intake, or ordinal variables modeled as a continuous variables.

Findings

Table 1 summarizes the PD factors associated with dietary vitamin C intake. Among the risk factors for Parkinson's disease, all but age (r=-0.019; p=0.09) were associated with vitamin C intake. Coffee drinkers and current smokers had significantly lower intake, while a history of plantation work was associated with lower vitamin C intake. Likewise, those reporting a bowel frequency of less than once per day had significantly lower vitamin C intakes. Total calories consumed was significantly associated with vitamin C intake (r=0.161; p<0l.001), but not PD risk

A bivariate model examining dose-response with vitamin C intake as a continuous variable on PD risk was statistically significant (p=0.035). However, an examination of the hazard ratio comparing the bottom quartile to consecutively higher quartiles suggest a threshold effect, with most of the increase in risk occurring in the highest quartile (HR=1.37; 95% CI 0.85-2.20; Table 2). When comparing the highest quartile to all the rest, the hazard ratio was of similar magnitude (1.33; 95% CI 0.93-1.90). However, after adjustment

for plantation work, current smoking, coffee intake and bowel frequency at the third examination, the association between high vitamin C intake and PD risk was attenuated (HR=1.17; 95% CI 0.80-1.73) and the test for trend was no longer significant. When supplement use was examined separately using data from the mail-out survey, no increased risk for PD was observed. Moreover, the observed hazard ratio was in the opposite direction (crude HR=0.99; 95% CI 0.95-1.03), indicating a slight, though non-significant protective effect.

Since the hazard ratio for vitamin C was not significant after adjusting for confounding variables, and supplement intake showed no increase in the risk for PD, we next examined whether fruit and fruit juice had a separate role in predicting PD. Each analysis of fruit intake simultaneously adjusted for the effects of smoking, coffee intake, age, plantation work, and bowel frequency on PD risk. High fruit consumption (more than three servings of fruit) as reported during the 24 hour recall interview at Exam 1 showed a significant increase in PD risk (adjusted HR=1.71, 95% CI 1.16-2.51). Fruit intake recorded during the food frequency questionnaire at Exam 1 showed a similar, but non-significant increases in PD risk (adjusted HR=1.55; 95% CI 0.83-2.89) among those reporting more than one serving of fruit per day. Likewise seven or more servings of fruit drink in the last week at Exam 3 showed a similar increase in PD risk (adjusted HR= 1.98; 05% CI 1.26-3.10).

Fruit and fruit drink consumption at Exams 1 and 3 were combined to create a summary indicator variables of usual fruit consumption. High fruit and fruit drink consumption was defined as one or more servings per day at Exam 1 or seven

or more servings in the last week at Exam 3. After adjustment for current smoking at Exam 3, coffee consumption, past plantation work, and bowel frequency, high fruit consumption at one or both examinations was associated with a hazard ratio of 1.98 (95% CI 1.33-2.93). The effect of fruit consumption on PD risk was similar for those with and without a history of plantation work (Table 3). When cases with early onset (≤75.4 years old, median age of onset) were excluded, the association between PD risk and high fruit/fruit juice intake at both exams was stronger (adjusted HR = 2.51; 95% CI 1.49-4.22); however, the relative risk for high fruit consumption as attenuated when only those with an earlier onset were included in the model (adjusted HR = 1.51; 95% CI 0.81-2.81). The indicator variable combined total servings from both food frequency questionnaires. When treated as a continuous variable to test for trend, this summary indicator was highly significantly associated with increased PD risk ($p < 0.001$). It is noteworthy that none of the variables estimating vegetable consumption at either of the first examination (raw or cooked vegetables) or the third examination (corn, tomato slice, tomato juice, lettuce and celery) were associated with increased PD risk. To test whether the increased consumption might reflect a predilection for sweets, as suggested by Hellenbrand [23] and Scheider [4], total sugar intake in grams from the Exam 1 24-hour recall was included in a model adjusted for age, coffee intake, and current smoking. Likewise, total consumption of sweets and dessert items (e.g., plain cake, Danish pastry, jelly/jam, pudding, ice cream, pastry, sugar added to coffee/tea) were also examined for an association with PD risk. Neither total of sugar intake (adjusted HR= 1.00 per gram; 95% CI 0.99-1.01) or total consumption of sweets

and desserts (adjusted HR=0.96 per serving; 95% CI 0.91-1.00) were associated with increased PD risk.

Interpretation

This study observed an association between increased fruit and fruit drink consumption and increased risk of developing PD. Although increased PD risk was also associated with high dietary vitamin C intake, the observed relative risk was no longer statistically significant after controlling for age, bowel frequency, smoking and coffee drinking. Since supplemental vitamin C did not appear to be associated with PD risk, these collective observations suggest that fruit consumption, rather than vitamin C intake, is associated with increased PD risk.

There are several possible explanations for these findings. One explanation may be that some of the fruits consumed may contain exogenous (e.g., pesticides, herbicides) or endogenous (e.g., tetrahydroisoquinoline) neurotoxins. It is possible that some of the fresh fruit consumed could contain residual pesticides. An earlier study of this cohort reported increased risk among plantation workers, presumably from increased exposure to pesticides [24]. Alternatively, endogenous toxins may be present in many fruits consumed in Hawaii, particularly tropical fruits. Several case control studies have also reported increased fruit consumption was associated with PD and other neurodenerative disorders. A study in the Caribbean reported a very strong association between tropical fruit consumption and progressive supranuclear palsy and atypical parkinsonism [1]. Although the type of fruit eaten was not recorded in the Honolulu Heart Program examinations, the tropical climate in Hawaii is an excellent environment for many of the same fruits implicated in the Caribbean study. Therefore, it is possible that

high consumers of fruit in general were more likely to also consume tropical fruits similar to those implicated in the Caribbean study.

A second possible explanation is that vitamin C itself may have neurotoxic effects, but error in the estimates of vitamin C intake may have attenuated the observed association, with a subsequent loss in statistical power. Several factors would have affected the reliability and validity of the estimated vitamin C intake, not the least of which was reliance on a single 24-hour recall to estimate usual diet. The calculation of vitamin C intake from that recall would also have been affected by individual differences in food preparation, the shelf life and storage of vitamin C containing foods, as well as industry variability in the fortification of fruit drinks and juices.

It is noteworthy that King et al. found significantly higher serum levels of ascorbic acid in PD cases than controls [25]. While this positive association may initially appear to contradict the free radical hypothesis of PD etiology, it could also be explained by the purported prooxidant activity of ascorbate. Several studies suggests that vitamin C can act as a pro-oxidant under certain cellular conditions, including the presence of free iron or copper [26]. Since the SN of persons with PD may contain excessive amounts of free iron in the cytosol, it is conceivable that vitamin C acts selectively as a prooxidant in this area of the brain. Furthermore, *in vivo* studies revealed that ascorbate can damage DNA and chromosomes in the murine stomach and liver after a single exposure [26]. Oxidative damage in each case was suppressed by catalase, an enzyme demonstrated to be deficient in parkinsonian brains [27]. Vitamin C has also been shown to facilitate neuronal firing and the release of neurotransmitters [20]; hence, high vitamin C concentrations could result in increased dopamine turnover and acceleration of autoxidation of dopamine, leading to formation of the free radical

formers such as 6-OHDA. Vitamin C has also been reported to modulate the binding of ligands to neural receptors including those of dopamine [28] and glutamate [29]. This enhanced receptor-ligand binding might also lead to an excitotoxicity (cell death resulting from over stimulation). There is evidence that 6-OHDA, MPTP, and even DA may have potential to act as excitotoxins [30].

Although the observation that supplemental vitamin C did not appear to increase PD risk apparently contradicts the hypothesis of vitamin C neurotoxicity, these data should be viewed with some caution. The data obtained on supplement intake were derived from a much smaller sample of surviving participants. Furthermore, the mail-out data were obtained relatively late in the study period (1988-1990), thus allowing for only a short follow-up period.

Finally, the association of PD with high levels of fruit, fruit drink, and dietary vitamin C intake could arise from confounding from other PD risk factors, or possibly from a differential detection bias among more health conscious non-smokers. For example, smoking confers an apparent protective effect against PD, the association between PD occurrence and vitamin C intake might reflect residual confounding resulting from non-differential recall of smoking behaviors among the HHP participants [31,32]. Although the association between vitamin C intake and PD risk was attenuated after adjustment for smoking, the association with fruit and fruit drink consumption was unaffected by statistical adjustment for confounding. Further evidence against that the observed association did not arise from confounding or a "health consciousness" –related detection bias was our observation that other "healthy" foods, such as vegetables, were not associated with increased PD risk. Moreover, although the supplement use data was limited, the slightly higher use among the unaffected participants seems

provide further evidence that the association did not arise as a result of healthier behaviors among non-smoking PD cases.

In summary, increased fruit and fruit drink consumption, rather than vitamin C intake, are associated with increased PD risk. The findings of this study, and other reports from our research group [24] suggest that environmental factors are associated with increased PD risk. Further investigation is needed to elucidate whether this increased risk is due to increased consumption of either endogenous plant borne toxins, or exogenous toxins such as pesticides or herbicides. Defining the role of food-borne toxins may provide further insights into the etiology and prevention of Idiopathic Parkinson's disease.

Table 1. Comparison of mean dietary vitamin C intake (mg), from a single 24-hour recall, for selected population characteristics Honolulu Heart Program, Examination I, 1965-1968.

Characteristic	No		Yes	
	N	Mean Intake	N	Mean Intake
Worked on a plantation	5347	111.9	2645	104.4
Current Smoker	4502	122.2	3502	93.0
Bowel frequency < 1/day	6506	111.6	1500	99.9
Coffee drinker	610	133.6	7396	107.4
Total Calories>2152 (median)	4263	97.1	3743	121.7
Age >53 years old (median)	4168	110.8	3838	107.9

Table 2. Inter-quartile estimates of relative risk ratios of Parkinson's disease for vitamin C intake, calculated from first examination 24 hour dietary recall interviews, Honolulu Heart Program, 1965-1993.

	Crude		**Adjusted***	
	Relative Risk	95 % Confidence Interval	Relative Risk	95 % Confidence Interval
first quartile	1.00	–	1.00	–
second quartile	1.21	0.71 - 2.03	1.03	0.62 - 1.71
third quartile	1.10	0.65 - 1.87	0.88	0.52 - 1.47
fourth quartile	1.45	0.88 -2.39	1.12	0.69 - 1.81

Fourth quartile vs. bottom three quartiles	1.33	0.86 –2.08	1.17	0.85 -1.73

* adjusted for current smoking, coffee drinking, age, bowel frequency, and history of working on a plantation

Table 3. Relative risk estimates of PD in men with high fruit and fruit juice intake as reported in Exam 1 24-hour recalls, and Exam 1 and Exam 3 food frequency questionnaires.

	Relative Risk	95 % Confidence Interval
24-hour recall of combined fruit intake	1.21*	0.98 - 1.50
High 24-hour fruit intake (>3 servings/day)	1.71*	1.16 - 2.52
High fruit intake from Exam 1 frequency questionnaire (>1 serving/day)	1.55*	0.83 - 2.89
High fruit drink intake from Exam 3 frequency questionnaire (≥7 servings/week)	1.98*	1.26 - 3.10
High combined fruit/fruit drink intake from Exams 1 and 3 frequency questionnaires	1.98*	1.33 - 2.93
Combined fruit/fruit drink and early age of onset (<75.4 years old)	1.51*	0.81 - 2.81
Combined fruit/fruit drink and later age of onset (≥75.4 years old)	2.51*	1.49 - 4.22
Combined fruit/fruit drink and history of plantation work	2.45†	1.33 - 4.52
Combined fruit/fruit drink and no history of plantation work	1.74†	1.03 - 2.93

*adjusted for current smoking, coffee drinking, age, bowel frequency, and history of working on a plantation

†adjusted for current smoking, coffee drinking, age, and bowel frequency

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Environmental, Life-Style, and Physical Precursors of Clinical Parkinson's Disease:

Recent Findings From the Honolulu-Asia Aging Study

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Running head: Precursors of clinical Parkinson's disease.

Key words: Parkinson's disease, risk factor, epidemiology.

Supported by a grant from the U.S. Department of the Army (DAMD17-98-1-8621), by the National Institutes of Health (National Institute on Aging contract N01-AG-4-2149 and grant 1-R01-AG17155-01A1, National Heart, Lung, and Blood Institute contract N01-HC-05102, and a National Institute of Neurological Disorders and Stroke grant 1-R01-NS41265-01), by the National Institute for Occupational Safety and Health (Contract HELD0080060), and by the Office of Research and Development, Department of Veterans Affairs. The information contained in this article does not necessarily reflect the position or the policy of the Government, and no official endorsement should be inferred.

Abstract

Background: Increased westernization with Japanese migration to the U.S. in the early 20th century is thought to have altered the risk of cardiovascular disease. Whether similar effects include changes in the risk of Parkinson's disease (PD) is not clear. This report describes the relations between environmental, life-style, and physical attributes and the incidence of PD that have been observed in the Honolulu-Asia Aging Study.

Methods: Beginning in 1965, environmental, life-style, and physical attributes were recorded at selected examinations in a cohort of 8,006 Japanese-American men. Subjects were followed for clinical PD.

Findings: During 30 years of follow-up, PD was observed in 137 men. Overall incidence (7.1/10,000 person-years) was generally higher than in Asia and similar to rates observed in Europe and the U.S. Precursors of PD included constipation, adiposity, years worked on a sugar or pineapple plantation, years of exposure to pesticides, and exposure to sugar cane processing. Factors showing an inverse association with PD included coffee intake and cigarette smoking. Among dietary factors, carbohydrates increased the risk of PD while the intake of polyunsaturated fats appeared protective. Total caloric intake, saturated and monounsaturated fats, protein, niacin, riboflavin, beta-carotene, vitamins A, B, and C, dietary cholesterol, cobalamin, α-tocopherol, and pantothenic acid showed no clear relation with clinical PD.

Interpretation: Findings suggest that several environmental, life-style, and physical attributes appear to be precursors of PD. Whether patterns of precursors can be used to identify individuals at high risk of future PD or can broaden the scope of early interventions or recruitment into neuroprotective trials warrants further study.

Introduction

Increased westernization with Japanese migration to the U.S. in the early 20th century is thought to have altered the incidence of cardiovascular disease through changes in diet, behavior, and the environment [1-6]. Whether similar effects include alterations in the risk of Parkinson's disease (PD) is not known, although worldwide differences in the incidence of PD suggest that geographic variation in unknown risk factor exposures may have a role in its etiology [7,8]. This report describes the relations between environmental, life-style, and physical attributes and the incidence of PD that have been observed in the Honolulu-Asia Aging Study.

Background and Resources

Study Sample

From 1965 to 1968, the Honolulu Heart Program began following 8,006 men of Japanese ancestry living on the island of Oahu, Hawaii for the development of cardiovascular disease [9-11]. At the time of study enrollment, subjects were aged 45 to 68 years. Initial screening included a baseline physical examination and documentation of cardiac and neurologic conditions to identify prevalent cases of cardiovascular disease. Additional follow-up included repeat examinations and the tracking of morbidity and mortality outcomes through a comprehensive system of surveillance based on a review of all hospital discharges, death certificates, and autopsy records. Within the Honolulu Heart Program, the Honolulu-Asia Aging Study was established in 1991 for dedicated research on neurodegenerative diseases and cognitive function in the elderly. Procedures were in accordance with institutional guidelines and approved by an institutional review committee. Informed consent was obtained from the study participants.

PD Case Finding and Diagnosis

In this report, 30 years of follow-up data were available on incident PD since the time of study inception (1965-1968). Prior to 1991, cases of PD were identified through a review of all hospital records for new and preexisting diagnoses of PD. Ongoing reviews also included a thorough evaluation of Hawaii death certificates and the medical records of local neurologists for cohort members suspected to have PD.

After 1991, study participants were screened for PD at examinations that occurred from 1991 to 1993. All subjects were questioned about a diagnosis of PD and the use of PD medications by a structured interview. Study participants received further screening by a technician trained to recognize the clinical signs of parkinsonism (including gait disturbance, tremor, and bradykinesia). Those with a history or sign of parkinsonism were referred to a study neurologist who administered standardized questions about symptoms and the onset of parkinsonism, previous diagnoses, and medication use, followed by a comprehensive and standardized neurologic examination. A diagnosis of PD was made by the study neurologists according to published criteria without access to the risk factor data examined in this report [12]. These required that the subject have the following: (1) parkinsonism (e.g., at least two of the four cardinal features: bradykinesia, rest tremor, rigidity, or postural reflex impairment); (2) a progressive disorder; (3) any two of a marked response to levodopa, asymmetry of signs, asymmetry at onset, or initial onset tremor; and (4) absence of any etiology known to cause similar features. Cases of parkinsonism related to progressive supranuclear palsy, multi-system atrophy, cerebrovascular disease, drug induced parkinsonism, post-encephalitic parkinsonism, or post-traumatic parkinsonism were not included among the cases of PD. During repeat exams that were given from 1994 to 1996 and from 1996 to 1998, subjects were again asked about a

diagnosis of PD and the use of PD medications. Medical records were further reviewed by the study neurologists who applied the same published criteria used earlier in making a diagnosis of PD [12]. Further description of the diagnosis of PD is described elsewhere [8,13].

Statistical Methods

After the measurement of an environmental, life-style, or physical attribute, age-adjusted incidence rates of PD in person-years were estimated according to various attribute levels [14,15]. All subjects were free of PD when follow-up began at the examination when an attribute was first observed. Independent effects of an attribute on the risk of PD were examined through the use of proportional hazards regression models [16]. Relative risks of PD were also estimated comparing the risk of PD between attribute levels. For analyses based on a small number of PD cases, p-values were estimated from permutation tests for exact logistic regression [17]. All reported p-values were based on two-sided tests of significance.

Findings

Among the men enrolled in the Honolulu-Asia Aging Study, the average age at the time of study inception (1965-1968) was 54 years (range: 45-68). In 30-years of follow-up, 137 developed PD (7.1/10,000 person-years). The average age at the time of diagnosis was 73 years (range: 54-89), and the average time to a diagnosis was 19 years (range: 2-30). Although we describe new observations from the Honolulu-Asia Aging Study, sample sizes and event counts may vary due to when follow-up began or through updated evidence that helps in the diagnosis of PD. In addition to the presentation of new data, we also expand on findings in earlier manuscripts from the Honolulu-Asia Aging Study based on sample sizes and event counts that were used in those original reports.

Cigarette Smoking and Coffee Intake

Data from a variety of sources suggest that smoking is protective against PD [13,18], although the biological basis that underlies the relation between smoking and PD is poorly understood. Identification of a protective effect of smoking is important since it could shed light on the unknown pathogenic mechanisms of PD along with similar relations that have been observed in Alzheimer's disease [19,20].

Prospective follow-up in the Honolulu-Asia Aging Study confirm that cigarette smoking is inversely related to the risk of clinical PD [13,18]. In the most recent report from Hawaii [18], 51% of all PD cases (52/102) occurred in 28% of the men who reported that they never smoked cigarettes. Among the 52 cases, only 19 would have been expected to occur had the risk of PD been similar to those who were former or current smokers. The association between smoking and PD is also unexplained by early mortality in men who smoked cigarettes and is independent of other factors that have been linked to PD, including the intake of coffee.

In addition to cigarette smoking, coffee has also been shown to have a protective effect on the risk of PD [13]. An effect further appears to be reproducible for different follow-up periods and with different methods of quantifying coffee intake (24-hour recall methods versus food frequency questionnaires). Based on 30 years of follow-up, nondrinkers of coffee experienced a 5-fold excess in the risk of PD as compared to men who consumed 28 oz/day or more (10.4 versus 1.9/10,000 person-years, respectively). The risk of PD also declined consistently with each increase in amount of coffee consumed ($p<0.001$). Among all PD cases, 31% (32/102) occurred in the 16% of men who reported that they were nondrinkers of coffee. Among the 32 cases, only 13 would have been expected to occur had the risk of PD been similar to those who consumed any amount of coffee.

For both cigarette smoking and coffee intake, effects are independent and strong. In the Honolulu-Asia Aging Study [13], the highest rate of PD occurred in men who neither smoked cigarettes nor drank coffee (15.1/10,000 person-years) as compared to an absence of PD in current smokers and those who consumed the most amount of coffee on a daily basis (≥28 oz/day). Although cigarette smoking reduced the risk of PD, there was a near constant dose-response relation between coffee intake and PD incidence for men who never smoked cigarettes, for those who were past smokers, and for those who were current smokers.

Plantation Work

In 1983, a description of parkinsonism in heroin addicts exposed to the neurotoxin MPTP intensified the search for environmental risk factors for PD [21]. MPTP is a contaminant contained in a synthesized recreational narcotic that has similarities in structure to the herbicide paraquat [22]. MPTP also has a toxic mode of action comparable to the insecticide rotenone [23]. Since these reports first appeared, special efforts have focused on identifying a role of agricultural chemicals in the etiology of PD. Subsequently, numerous case-control studies have found that well water, farming, rural living, and exposure to pesticides are associated with an increased risk of PD [24].

Political and social pressures that led to the migration of Japanese to the U.S. in the early 20th century help make the Honolulu-Asia Aging Study a valuable resource for the study of the relation between a constellation of factors associated with agriculture and the risk of PD. Cohort members were either immigrants or the progeny of immigrants from the same regions of Japan who migrated to Hawaii as contract laborers to serve in the sugar and pineapple industries. It provides a useful opportunity to examine the effects of a dominant and relatively homogeneous industry (plantation work) on the risk of PD.

Based on 30-years of follow-up, recent data have demonstrated that differences in the risk of PD are modest for men who spent 10 years or less as a plantation worker, while beyond 10 years, risk of PD nearly doubles [24]. For men who worked 10 years or less on a plantation, incidence of PD ranged from 5 to 6/10,000 person-years as compared to 10.3/10,000 person-years in those who worked more than 20 years (p=0.011). Although findings were based on accurate plantation work histories that were collected at the beginning of study inception (1965-1968), specific data on sugarcane and pineapple plantation exposures were not available.

Nevertheless, at repeat examinations that occurred 6 years into follow-up (1971-1974), participants received an additional exam where inquiries were made about nonspecific exposures to sugarcane processing that lasted for at least a year. Based on 24-years of follow-up after this exam, data suggest that sugarcane processing is associated with the risk of PD. As seen in figure 1, however, the association appears most apparent in men who did not smoke cigarettes. For nonsmokers, the incidence of PD was increased by nearly 5-fold in those exposed to sugarcane processing as compared to those who were not (bottom left panel of figure 1, p=0.008). Regardless of coffee drinking status, the corresponding risk was 2-fold (top of figure 1), although not statistically significant. For non-cigarette smokers, the effect of sugarcane processing on the risk of PD also appeared to be independent of years worked on a plantation. Additional interpretations suggest that cigarette smoking is associated with a reduced susceptibility to PD that might otherwise be attributed to sugarcane processing.

Exposure to Pesticides

Findings of an association between plantation work and the risk of PD in the Honolulu sample [24] has further suggested that increasing years of exposure to pesticides also elevates the risk of PD, although results were not statistically significant (p=0.101). As with sugarcane

processing, however, additional analyses indicate that cigarette smoking reduces the susceptibility to PD associated with pesticide exposure. Reduced susceptibility also seems to occur for coffee drinkers.

As seen in figure 2, risk of PD in men who drank coffee (top right panel) or smoked cigarettes (bottom right panel) appeared unrelated to years of exposure to pesticides. In the absence of these factors, however, susceptibility to pesticides seems to increase. Among nondrinkers of coffee, risk of PD was 3-times higher in men who were exposed to pesticides for more than 3 years (63.4/10,000 person-years) as compared to men with no exposure to pesticides (21.4/10,000 person-years, p=0.044). Risk of PD in nonsmokers also seemed to increase susceptibility to pesticides for exposures beyond 3 years versus men who were not exposed (27.4 versus 11.8/10,000 person-years, p=0.053). While interactions were not statistically significant, such findings suggest that the risk of PD may have multi-factorial origins and variations in susceptibilities.

Unfortunately, the data in figure 2 are based on self-reported exposures to pesticides at either work or at home. While reported responses can be quite variable, documentation of home exposure is difficult since it depends on individual recall and knowledge about product contents and cumulative exposure experiences. Regular exposure to pesticides at work may also have been more common than perceived, and many who reported not being exposed could have had high levels of exposure.

In response to these issues, exposure to pesticides was independently estimated using occupation and industry codes created by the U.S. Bureau of the Census [25] that were collected among the study participants at the time of study inception (1965-1968). Through resources available at the U.S. National Institute for Occupational Safety and Health, a measure of

exposure was assigned to each occupational and industrial code combination with the following definitions: 0=none, 1=low, 2=moderate, and 3=high. In addition to data on usual occupation and industry, years spent in these occupations and industries were also collected. Based on these additional data, an overall measure of intensity to pesticide exposure was created by multiplying the exposure associated with an occupational and industrial code combination (0, 1, 2, or 3) by the number of years spent in that job related combination. The average value of the overall intensity measure was 4.6 (range: 0 to 153).

Based on the occupation and industry work histories, an association between pesticide exposure and the risk of PD appears to be confirmed (figure 3). As with the self-reported measure, susceptibility to PD seemed reduced in men who smoked cigarettes or drank coffee. For nondrinkers of coffee, however, there is a near linear increase in the incidence of PD with increasing intensity of pesticide exposure ($p=0.009$). A similar trend also seems to occur in nonsmokers, although it is not statistically significant ($p=0.084$).

Constipation

Since the time of James Parkinson, constipation has been known to be common in patients with PD [26]. Recent data suggest that up to 80% of PD patients are afflicted with constipation [27], and some believe that defacatory dysfunction could be associated with PD severity and duration [28]. Although subject to uncertain recall of constipation histories, two case reviews further suggest that constipation may predate PD. In one series, 178 PD patients were asked to recall their bowel habits prior to the diagnosis of PD. Among this group, 46% reported having constipation, while in spouse controls (largely women), 28% had complaints of constipation [29]. In another report, constipation was reported to have occurred prior to a diagnosis of PD in 10 of 12 patients by an average of 16 years [30].

Recently, the Honolulu-Asia Aging Study has more clearly demonstrated that constipation predates PD based on 24 years of follow-up after data were first collected on bowel movement frequency at examinations that occurred from 1971 to 1974 [31]. A major strength of this finding is that it is based on the collection of bowel movement patterns following a standardized research protocol well before the development of PD. Here, age-adjusted incidence declined consistently from 18.9/10,000 person-years in men with <1 bowel movement/day to 3.8/10,000 person-years in those with >2/day (p=0.005). Use of cigarettes and coffee intake failed to alter the association between bowel movement frequency and the risk of PD.

Data further suggest that the greatest risk of PD is likely to occur when constipation is resistant to treatment. In the Honolulu sample, the age-adjusted risk of PD was highest (51.6/10,000 person-years) in the cohort of men who reported using laxatives at least 2 times per week and continued to have <1 bowel movement/day (see figure 4). Among heavy users of laxatives, rates of PD declined as bowel movement frequency increased (p=0.009), suggesting that the type of constipation associated with PD (unresponsive to therapy) is unique. This seems reasonable since most constipation is unrelated to PD.

Body Fat Distribution

While loss in body fat is common in patients with clinical PD [32,33], reported findings based on cross-sectional and case-control studies (with uncertain recall and timing of anthropometric histories) are far from clear. In a recent mouse study with genetically induced obesity, there was an increased vulnerability to the neurotoxicants methamphetamine and kainic acid through reductions in levels of striatal dopamine and tyrosine hydroxylase and to elevated levels of glial fibrillary acidic protein, a sensitive indicator of neuronal damage [34]. Evidence for an effect of complex nervous system interactions involving autonomic dysfunction on

appetite regulation and energy metabolism [35], and recent observations that obesity in humans is related to the depletion of striatal dopamine receptor availability, suggests that nigrostriatal system disorders have associations with both PD and adiposity [36].

To help address this issue more clearly, the Honolulu-Asia Aging Study was able to access archived data on body composition that was collected at more than one physical examination following standardized procedures of measurement [37]. Based on measurements of body mass index (BMI), subscapular skinfold thickness (SSF), and tricep skinfold thickness (TSF) at the time of study enrollment (1965-1968), the leanest group of men were found to experience the lowest incidence of PD over 30-years of follow-up. Among the measures of adiposity, age-adjusted incidence of PD increased consistently by three-fold from 3.7/10,000 person-years in the bottom quartile of TSF (1-5 mm) to 11.1/10,000 person-years in the top quartile (11-32 mm, $p<0.001$). Associations of TSF with PD were also independent of cigarette smoking, coffee consumption, physical activity, daily caloric and fat intake, and the other measures of adiposity ($p<0.001$). While rates of PD were lowest in the bottom quartile of BMI and SSF versus higher quartiles, associations with PD were weaker than they were for TSF. The association of TSF with clinical onset before age 65 years was similar to that observed in later life. Neither cigarette smoking nor coffee intake reduced the susceptibility to PD that was associated with an elevated TSF.

In addition to levels of adiposity observed in middle adulthood [37], those that were measured in later life also appeared to be related to the risk of clinical PD (see figure 5). During a repeat physical examination that was given from 1991 to 1993, measurements of BMI, SSF, and TSF were available in 3,512 surviving members of the original cohort aged 71 to 93 years. In the remaining 5 to 7 years of follow-up, 27 men developed PD (20.3/10,000 person-years).

Age-adjusted incidence of PD for men in the bottom quintile of TSF (2-6.5 mm) was 8.8/10,000 person-years versus 34.5/10,000 person-years in those in the top quintile (13-30 mm).

Although it might be expected that the small number of PD cases would limit statistical power, the incidence of PD continued to rise significantly with increasing TSF ($p=0.005$). Effects also remained significant after adjustment for age, BMI, and SSF ($p=0.013$). As with adiposity measures observed in mid-life, associations between BMI and SSF were not statistically significant. These findings further suggest that the association between adiposity and PD observed in middle adulthood also extends to the elderly.

Dietary Intake

Studies of the relation between diet and PD often report conflicting results. Most are based on case-control designs with the usual limitations involving uncertain recall of past dietary behaviors. In one case-control study, comparisons were made between dietary histories using food-frequency questionnaires [38]. Patients with PD were found to have consumed higher levels of carbohydrates and lower amounts of beta-carotene and niacin prior to disease onset than controls. There were no apparent associations observed between protein and fat intake and PD. In contrast, others report that higher caloric and fat intake consumed during the year prior to study enrollment are associated with PD, while there was no association with carbohydrates [39]. Based on measures of dietary habits followed during most of adult life, an increase in the intake of animal fat and vitamin D was described in patients with PD versus matched controls [40].

Similar dietary data were collected in the Honolulu-Asia Aging Study at the time of study enrollment (1965-1968) with 30 years of follow-up for the first appearance of clinical PD. Here, nutrient intake was determined by a dietitian based on 24-hour recall methods and validated against a full week of dietary records in a subset of the original cohort. Comparisons between the

two assessment methods showed no significant differences between the instruments for measuring dietary intake, and day-to-day variation was less than typical in western cultures [41]. While errors in recall are less of an issue here, it is likely that other errors in measurement (also shared with case-control studies) are not entirely removed. For example, 24-hour recall may not reflect typical dietary patterns, and some groups, particularly obese individuals, often under-report true dietary intake [42,43]. Nevertheless, these types of studies are often considered to be the best available. In the presence of the errors in data collection in dietary surveys, it is likely that the observed effects of food intake on disease provide an underestimate of true associations. Unfortunately, because of the high level of correlation that exists among dietary intake variables, identifying specific relations is extremely difficult in any cohort or case-control study.

Based on the calculation of micronutrient intake from the 24-hour recall data in the Honolulu-Asia Aging Study, there is some consistency with associations that have been reported elsewhere, while most appear to be absent. Among the latter, total caloric intake, protein, niacin, riboflavin, beta-carotene, vitamins A, B, and C, dietary cholesterol, cobalamin, α-tocopherol, and pantothenic acid had no clear relation with clinical PD. Although the intake of vitamin E in the Honolulu-Asia Aging Study was modestly related to a reduced odds of PD, legumes (a food rich in vitamin E) were associated with a marked protective effect [44]. Associations appeared for other dietary variables, but most consistently in subjects who were nonsmokers and nondrinkers of coffee. Further work in this area is ongoing in the Honolulu-Asia Aging Study.

Among the associations identified thus far, intake of carbohydrates and polyunsaturated fats appear to have the most consistent relation with the risk of PD. Associations observed in the Honolulu-Asia Aging Study are described in figure 6 for those who were nondrinkers of coffee (top 2 panels) and in those who reported never smoking cigarettes (bottom 2 panels). Here, the

age-adjusted incidence of PD is plotted by median intake values within quintile ranges of the daily intake of carbohydrates (left 2 panels) and polyunsaturated fat (right 2 panels) based on dietary intake that was observed at the time of study enrollment (1965-1968). For carbohydrates (left 2 panels), PD incidence rose significantly with increasing intake for both non coffee drinkers and never smokers ($p<0.05$). Differences in the risk of PD, however, were modest up to the 4th and 5th quintiles of carbohydrate intake. In contrast, the intake of polyunsaturated fats appeared protective against PD, particularly in men who never smoked cigarettes ($p=0.042$). For those who were never smokers of cigarettes, the effects of carbohydrates and polyunsaturated fats were also independent of each other. Saturated and monounsaturated fates were unrelated to the risk of PD in this sample of men.

Discussion

While geographic variation in the incidence PD is consistent with an environmental role in the development of PD, more convincing evidence is based on differences in the risk of PD that have been observed to occur with migration. For example, migration from Asia and western Africa to the U.S. has resulted in an increase in the incidence of PD within these ethnic communities as compared to reported rates from countries of origin [7,8]. The incidence of PD in the Japanese-American men enrolled in the Honolulu-Asia Aging Study is also higher than in Japan and are typical of rates that have been observed in Europe and the U.S. Although difficulties in how PD is defined can contribute to these differences, findings from the current report suggest that a role of environmental, life-style, and physical attributes on the risk of PD is real. Specifically, observations suggest that precursors associated with PD can include coffee intake, cigarette smoking, plantation work, exposure to pesticides, constipation, body fat distribution, and possibly diet.

Although associations between these precursors and PD are important, it is also noteworthy to draw attention to the long delay from the time of precursor measurement to the time of diagnosis of clinical PD. In many instances, delays in diagnosis exceeded 15 years after risk factor measurement [37]. Such long latency periods are in contrast to the estimated 3 to 6 year preclinical periods based on findings from neuroimaging and neuropathology studies [45,46]. While further explanation is needed, the long interval between precursor measurement and the diagnosis of PD may provide some insights into the pathogenesis of PD and to Lewy body formation that can begin as early as 25 years of age [47]. The possibility that PD neuropathology has origins in early life suggests that PD progression is slower and more subtle than previously thought. Based on an increased risk of PD due to long-term exposures to pesticides and plantation work in the Honolulu-Asia Aging Study, this may also mean that the development of PD is not inevitable if exposures can be limited or removed. It further suggests that prevention of PD could begin in early adulthood.

Explanations for the observed relations between a precursor and PD are unclear. It must first be noted that the term precursor does not imply that factors associated with PD are casual or are the result of processes leading to PD. At the very best, findings merely suggest that these factors can predate a diagnosis of clinical PD. In some instances, cumulative exposure to a precursor during early life may contribute to increased PD risk indirectly by increasing susceptibility to other factors that cause PD in later life.

In other cases, the effects of these precursors on PD progression could be more direct. Dietary intake of antioxidants could reduce oxidative stress and free-radical damage to neurons in the substantia nigra. Others have suggested that toxic levels of iron and manganese promote oxidative stress [40]. Effects of coffee and cigarette smoking could be important by modulating

neurotransmitter and neuroreceptor systems in the brainstem and corpus striatum or by directly interfering with the uptake of neurotoxins [13,18]. Findings of an association between pesticides and PD by two methods of quantification are consistent with the growing evidence for a neurotoxic role of pesticides on selective nigral injury, Lewy body formation, and responses to levodopa [24].

Based on the observation that coffee intake and cigarette smoking seem to reduce the susceptibility to PD due to other precursors, findings further support the possibility that a high risk of PD requires exposure to a combination of factors. Genetic susceptibility may also have an important role. The complex interaction among a constellation of these factors and their role in PD development may offer a partial explanation for why identification of risk factors for PD has been illusive. Although discouraging, this also suggests that reduced exposure to any single precursor could sufficiently delay or eliminate neuropathologic processes that lead to PD through the requirement that precursors need to coexist for PD progression to continue.

Whether combinations of precursors, particularly cigarette smoking, coffee intake, and possibly constipation histories can be used as enrollment criteria for the study of PD deserves consideration. Such design strategies could increase the potential for maximizing therapeutic effects in a clinical trial. It might also seem reasonable that in prospective cohort studies of precursors of PD, that focus should be on those groups where risk is highest, where accrual of events is quicker, and lengths of follow-up can be reduced. Identifying collections of precursors for PD (in combination with a family history and emerging movement abnormalities) could also lead to more effective strategies for identifying early or suspected disease, as well as provide for different approaches to prevention and intervention.

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Figure Legend

Figure 1: Age-adjusted incidence of PD according to exposure to sugar cane processing for at least 1 year as reported at physical examinations received from 1971 to 1974 within coffee drinking and cigarette smoking strata.

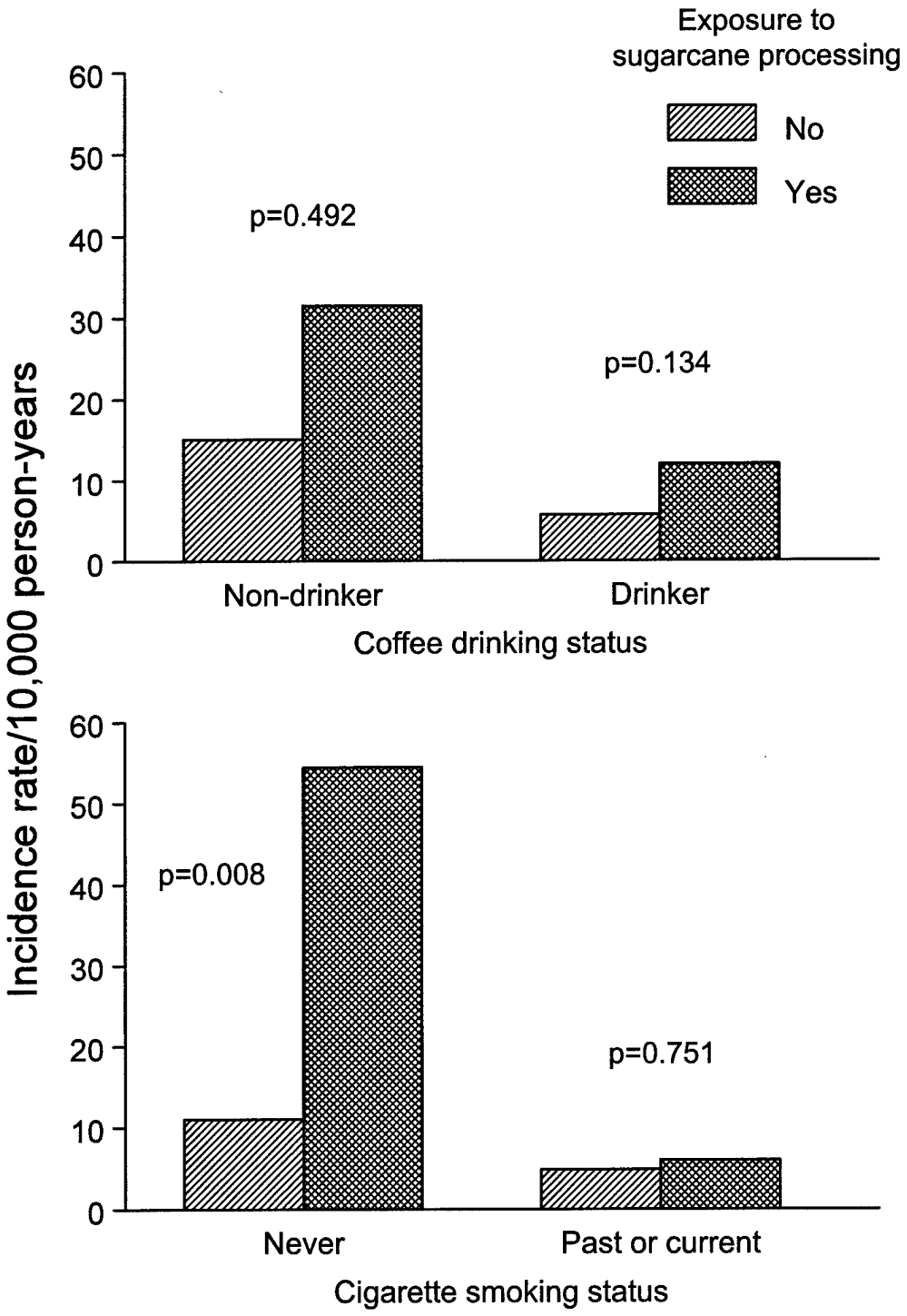
Figure 2: Age-adjusted incidence of PD according to self-reported years of pesticide exposure reported at physical examinations received from 1971 to 1974 within coffee drinking and cigarette smoking strata.

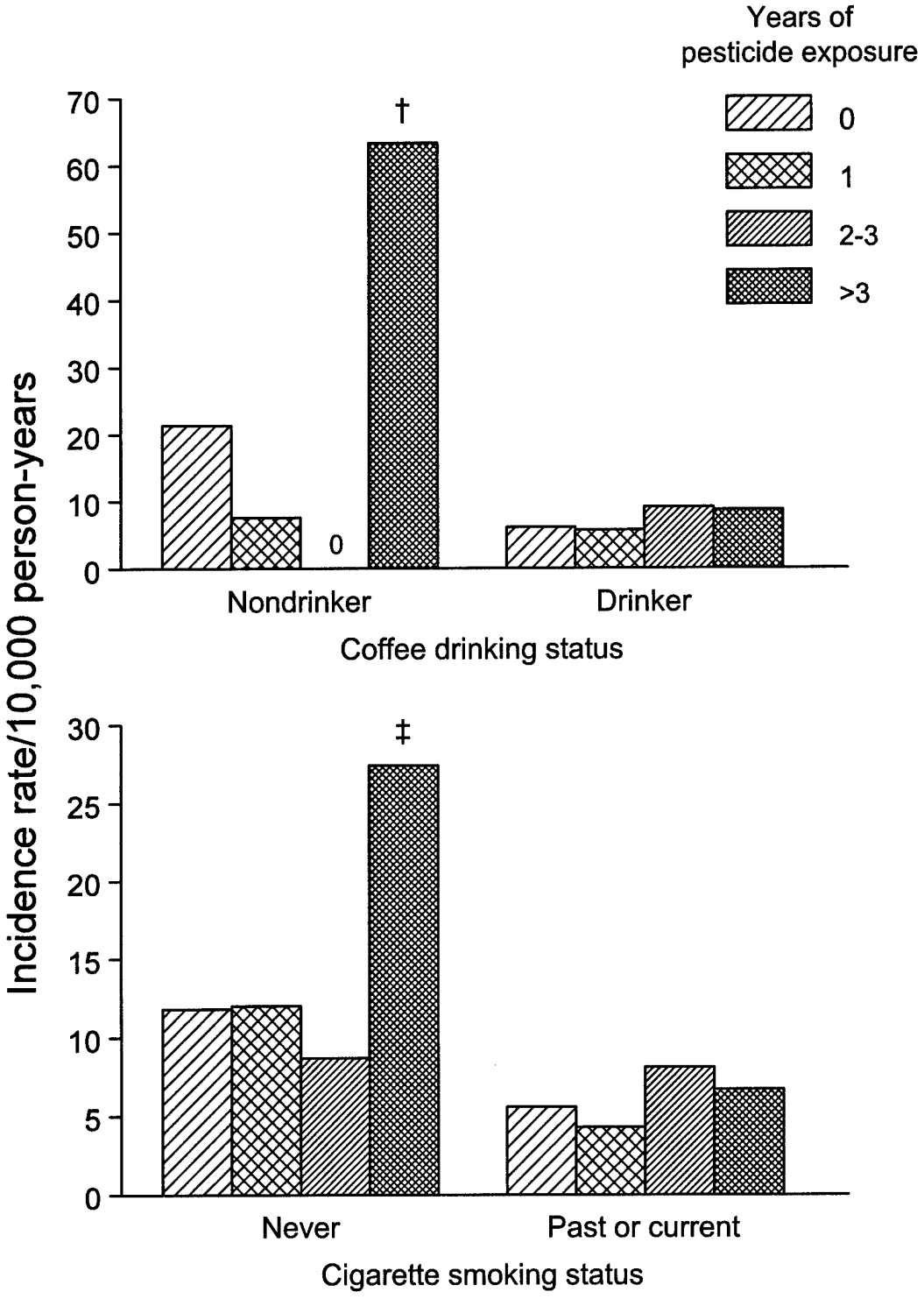
Figure 3: Age-adjusted incidence of PD according to intensity of pesticide exposures associated with industrial and occupational codes recorded at physical examinations received at the time of study enrollment (1965-1968) within coffee drinking and cigarette smoking strata.

Figure 4: Age-adjusted incidence of PD according to bowel movement frequency and the use of laxatives reported at physical examinations received from 1971 to 1974. P-values represent a test for trend based on modeling bowel movement frequency as a continuous variable.

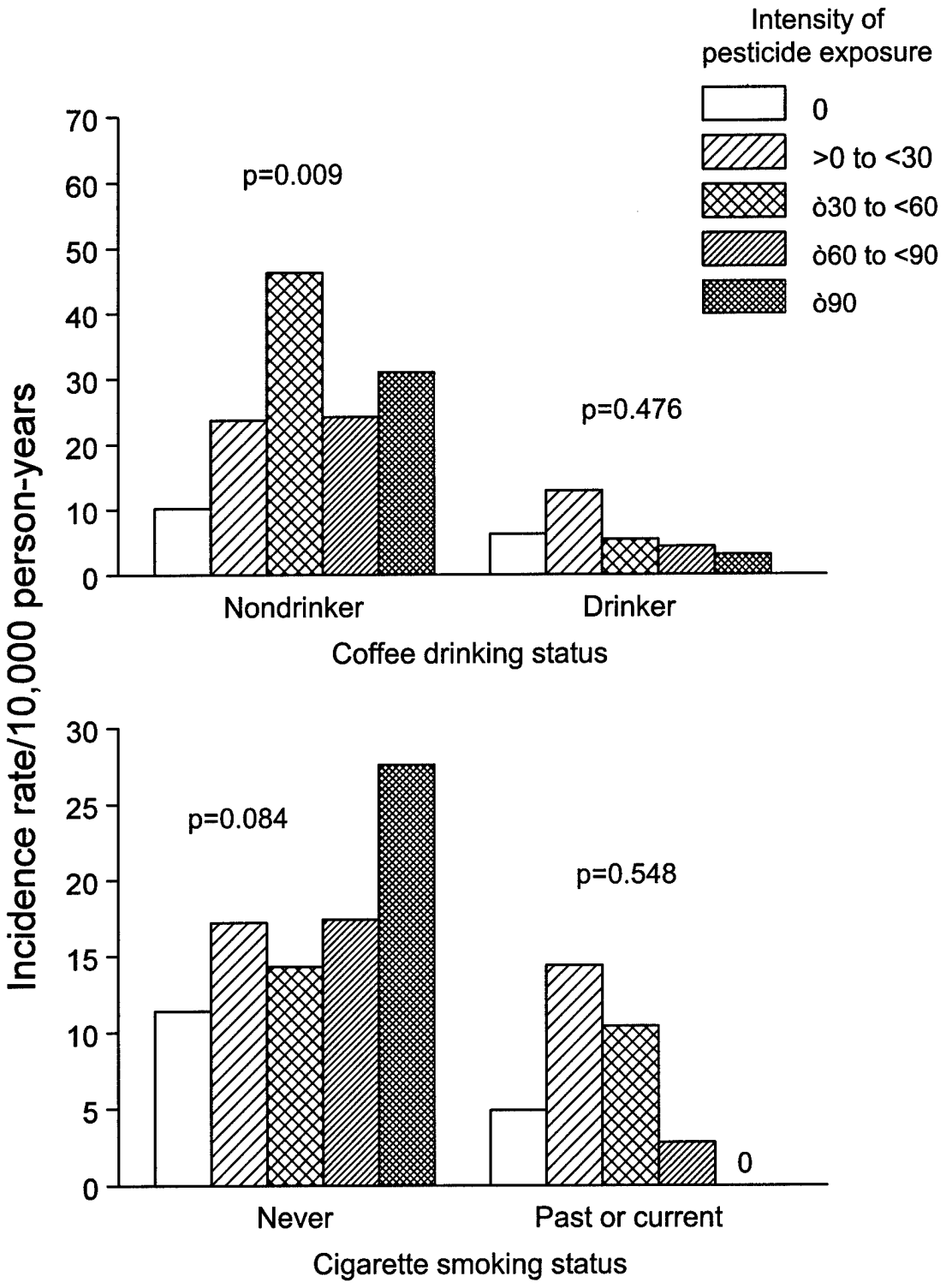
Figure 5: Age-adjusted incidence of PD by median levels of tricep skinfold thickness within quintile ranges in men aged 71 to 93 years at physical examinations received from 1991 to 1993. P-values represent a test for trend based on modeling tricep skinfold thickness as a continuous variable.

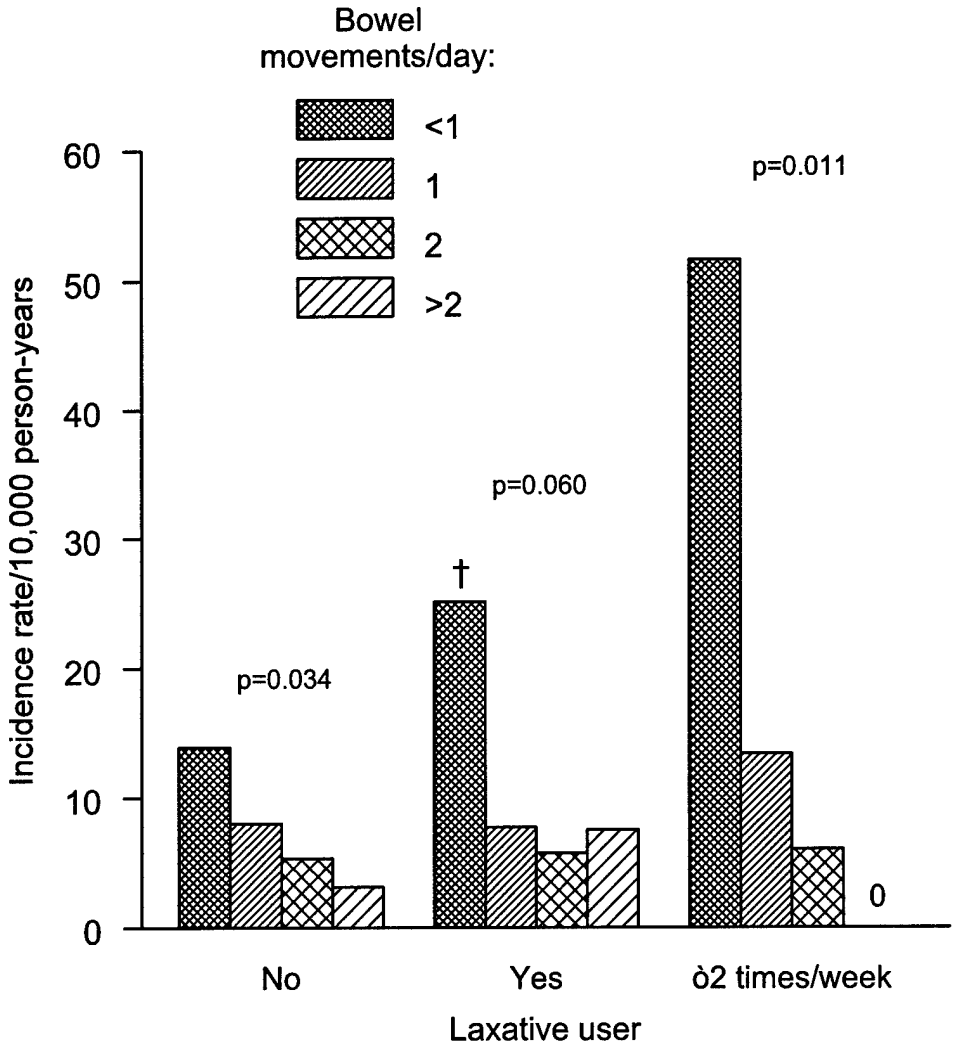
Figure 6: Age-adjusted incidence of PD by median intake values within quintile ranges of daily intake of carbohydrates, iron, manganese, and pyroxidine at the time of study enrollment (1965-1968). Subjects were nonsmokers and nondrinkers of coffee. P-values represent a test for trend based on modeling each intake value as a continuous variable.


Exposure to
sugarcane processing
No
Yes
Incidence rate/10,000 person-years
60
50
40
30
20
10
0
p=0.492
p=0.134
Non-drinker
Drinker
Coffee drinking status
p=0.008
p=0.751
Never
Past or current
Cigarette smoking status

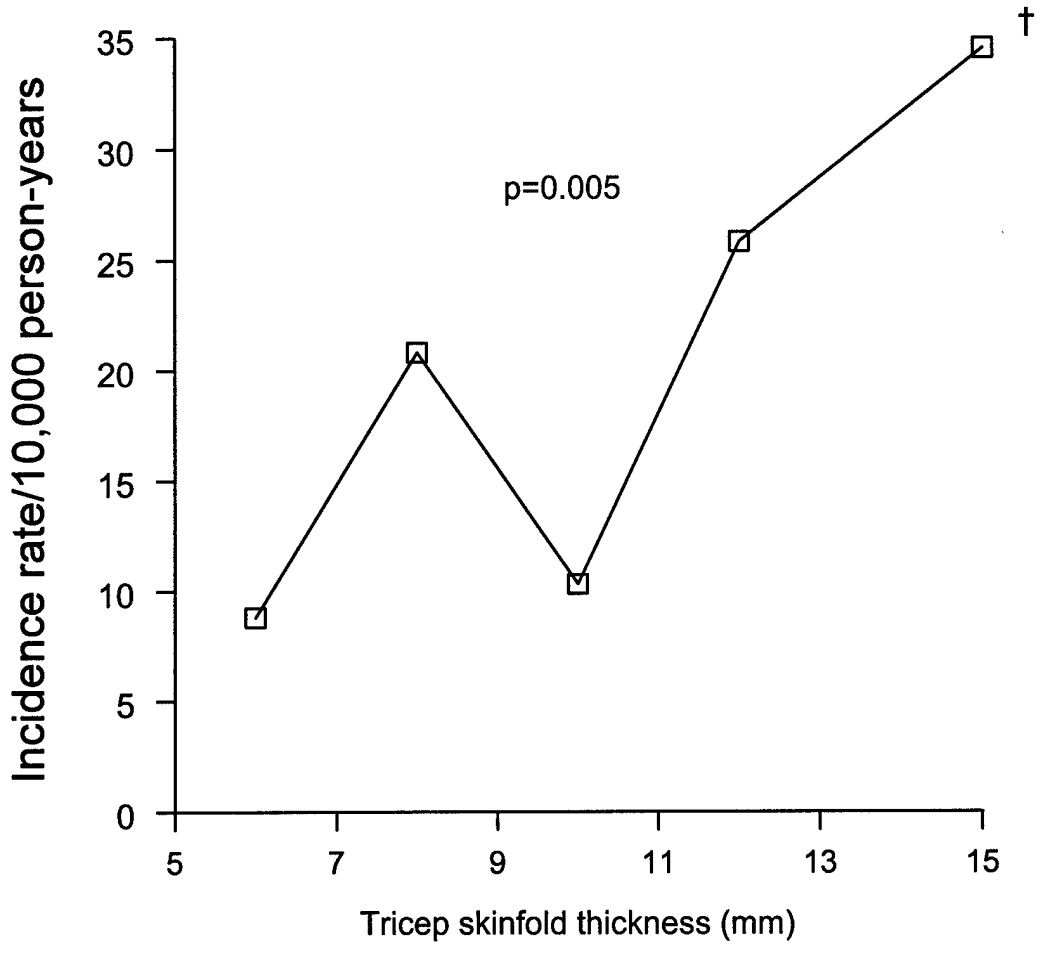


Significant excess versus unexposed men: †p=0.044, ‡p=0.053

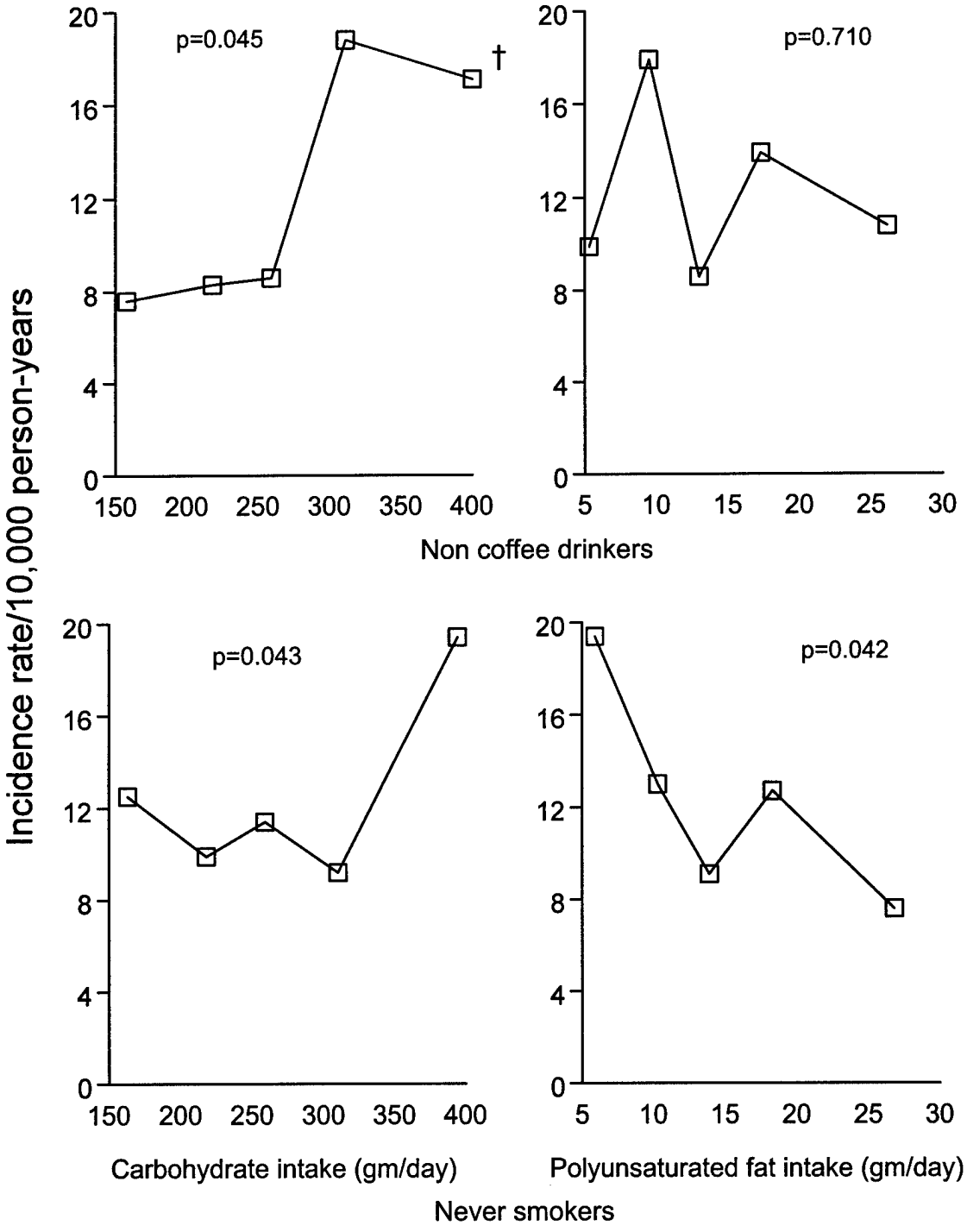

Intensity of pesticide exposure
0
>0 to <30
ò30 to <60
ò60 to <90
ò90
Incidence rate/10,000 person-years
70
60
50
40
30
20
10
0
p=0.009
p=0.476
Nondrinker
Drinker
Coffee drinking status
30
25
20
15
10
5
0
p=0.084
p=0.548
0
Never
Past or current
Cigarette smoking status



†Significant excess of PD vs. men with more frequent bowel movements (p=0.009).



†Plotted according to median tricep skinfold thickness within a quintile.



†Plotted according to median intake within a quintile.

APPENDIX D

ORIGINAL CONTRIBUTION

Plantation Work and Risk of Parkinson Disease in a Population-Based Longitudinal Study

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Context: Parkinson disease (PD) has an unknown cause; however, convincing evidence is emerging that indicates pesticides can selectively injure the dopaminergic system in laboratory animals. Retrospective studies in humans demonstrate a link between exposure to agricultural lifestyle factors and PD.

Objective: To determine whether working on a plantation in Hawaii and exposure to pesticides are associated with an increased risk of PD decades later.

Design and Setting: Prospective cohort study based on the island of Oahu, Hawaii, with 30 years of follow-up. Years of work on a plantation were assessed by questionnaire at study enrollment in 1965. Self-reported information on pesticide exposure was collected at a separate examination 6 years later.

Participants: Participants were 7986 Japanese American men born between 1900 and 1919 who were enrolled in the longitudinal Honolulu Heart Program.

Main Outcome Measures: Incident PD was determined by medical record review or by an examination conducted by a study neurologist at a later date.

Results: During follow-up, 116 men developed PD. Age-adjusted incidence increased significantly among men who worked more than 10 years on a plantation. The relative risk of PD was 1.0 (95% confidence interval, 0.6-1.6), 1.7 (95% confidence interval, 0.8-3.7), and 1.9 (95% confidence interval, 1.0-3.5) for men who worked on a plantation 1 to 10 years, 11 to 20 years, and more than 20 years compared with men who never did plantation work ($P=.006$, test for trend). Age-adjusted incidence of PD was higher in men exposed to pesticides than in men not exposed to pesticides although this was not statistically significant ($P=.10$, test for trend).

Conclusion: These longitudinal observations regarding plantation work in Hawaii support case-control studies suggesting that exposure to pesticides increases the risk of PD.

Arch Neurol. 2002;59:000-000

THE CAUSE of Parkinson disease (PD) is unknown. There is no treatment that prevents the disease or slows progression, and there are no confirmed modifiable risk factors. However, the description in 1983 of parkinsonism secondary to exposure to the protoxin MPTP (*N*-methyl-4-phenyl-1, 2,3,6-tetrahydropyridine) intensified the search for environmental risk factors.[1] The chemical structure of MPP+ (1-methyl-4-pyridinium), the toxic metabolite of MPTP, is similar to the herbicide paraquat.[2] Additionally, the toxic mechanism of action of MPP+, inhibition of mitochondrial respiration at complex I, is similar to that of the insecticide rotenone.[3] Supporting a possible role for these compounds in the cause of PD are recent reports of decreased motor activity commensurate with dopamaminergic system damage in rats given rotenone and mice given paraquat and the dithiocarbamate fungicide maneb in combination.[2,3] In humans, there are reports of increased levels of the organochlorine compound dieldrin in brains of patients with PD compared with healthy controls and controls with Alzheimer disease.[4,5] These discoveries have focused suspicion on exposure to agricultural chemicals as a risk factor for PD.

Numerous case-control studies in humans have found well water drinking, farming, rural living, and exposure to pesticides and herbicides to be associated with an increased risk of PD.[6-15] Although these findings have been consistent, retrospective assessment of exposure can be subject to recall bias. In this article, prospectively collected data about sugarcane and pineapple plantation work among participants in the Honolulu Heart Program are used to examine the

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relationship of midlife years of plantation work with incident PD in late life.

METHODS

POPULATION AND STUDY DESIGN

The Honolulu Heart Program began in 1965 with examination of 8006 men of Japanese ancestry, 45 to 68 years old, living on the island of Oahu, Hawaii. The initial examination consisted of face-to-face interviews and physical evaluation. Demographic, dietary, and health status data were obtained.[16,17] There are 36 years of follow-up with continued hospitalization and death record surveillance. Follow-up examinations were performed from 1968-1970, 1971-1974, 1991-1993, and 1994-1996. Research on neurodegenerative diseases of aging began in 1991 with establishment of the Honolulu-Asia Aging Study. An institutional review committee approved the procedures; informed consent was obtained from all participants. Details regarding study design were previously published.[18-20]

PD CASE FINDING AND DIAGNOSIS

For this article, 30 years of follow-up data were available. Incident cases of PD were identified through 4 sources.[21] Sources prior to 1991 were (1) review of all cohort member's hospitalization records for all diagnoses of PD after 1965, (2) ongoing review of all Hawaiian death certificates, and (3) review of medical records of all patients with PD from the offices of local neurologists cross-checked with the cohort member list.[18,21]

After 1991, diagnosis of PD was based on complete reexaminations of the entire cohort from 1991-1993 and 1994-1996. During the 1991-1993 examination,[21] all subjects were questioned about a history of PD and PD medications. Subjects with a history of PD or parkinsonism symptoms or signs were referred to a study neurologist (G.W.R. and J.S.P.) who administered standardized questions about symptoms and onset of parkinsonism, previous diagnoses, and medication usage, followed by a comprehensive and standardized neurological examination including the Unified Parkinson's Disease Rating Scale.[22] Diagnosis of PD was based on consensus from at least 2 neurologists (G.W.R., C.M.T., and J.S.P.) according to published criteria.[23] These required that the subject have (1) parkinsonism, (2) a progressive disorder, and (3) any 2 of the following: a marked response to levodopa treatment, asymmetry of signs, asymmetry at onset, or initial onset tremor. Cases of parkinsonism related to other neurodegenerative disorders, cerebrovascular disease, medications, trauma, or postencephalitic parkinsonism were not included among the cases of PD. Additional cases of PD were identified during the 1994-1996 examination through structured interviews inquiring about a history of PD or PD medications. A study neurologist (G.W.R.) confirmed these cases by medical record review and application of the above criteria.

Age at diagnosis was used instead of age at onset to avoid inaccuracies associated with recall of symptom onset for a chronic disease with gradual onset. Two prevalent cases of PD identified at the 1965-1968 examination were excluded from analysis.

YEARS WORKED ON A PLANTATION AND OTHER VARIABLES

When follow-up began (1965-1968), study participants were asked if they ever had a regular job on a plantation and for how many years. Among the 8004 men without PD, responses were collected from 7986. No differentiation was available between sugarcane and pineapple plantations. Intensity of exposure was relatively accurate since subjects were asked only about holding a regular job on a plantation and the number of years. At a follow-up examination 6 years after the baseline examination (1971-1974), fieldwork was further broken down by work on sugar and pineapple plantations. However, self-reported years of work on either type of plantation combined regular employment with sporadic and part-time work, making it difficult to quantify the regularity and intensity of exposure. For this reason primary analyses presented in this article use the combined measure of either sugarcane or pineapple plantation work collected at the baseline examination. Secondary analyses also use the less accurate exposure data broken down by type of plantation available at the follow-up examination.

At the same 1971-1974 examination, participants were asked about exposure to pesticides for at least 1 year at home or at work. Duration of exposure was collected by asking about mean days per year of exposure, age exposure started and stopped, and number of nonoverlapping intervals of exposure. Years of pesticide exposure were then calculated by summing total days of exposure across all nonoverlapping years of exposure duration. Data on pesticide exposure were available for 6854 men, about 90% of the surviving members of the original Honolulu Heart Program cohort.

Information on other potentially confounding variables collected at the beginning of follow-up included age, pack-years of cigarette smoking, and intake of coffee. Cigarette smoking and intake of coffee and caffeine have previously been shown to be associated with a decreased risk of PD in this cohort of men,[18,24] and analyses of plantation work were adjusted for these covariates.

STATISTICAL ANALYSIS

Crude and age-adjusted incidence rates of PD in person-years were estimated according to years worked on a plantation based on 30 years of follow-up available in the sample of 7986 men.[25] Similar person-year rates of PD were also estimated across years of pesticide exposure based on the remaining 24 years of follow-up in the 6854 men in whom such data on exposure to pesticides were collected 6 years later (1971-1974). Age-adjusted risk factor comparisons across ranges of years worked on a plantation are also provided based on analysis of covariance procedures.[25] Proportional hazards regression models were used to test for effect of years worked on a plantation and years of pesticide exposure on risk of PD.[26] Effects were also estimated after adjusting for age, pack-years of cigarette smoking, and daily intake of coffee. Years worked on a plantation and years of pesticide exposure were modeled as continuous variables comprising a test for trend or a dose-response relationship between plantation work and pesticide exposure and the risk of PD. Relative risks of PD (and associated confidence intervals [CIs]) were also estimated comparing the risk of PD in men who worked various amounts of time on a plantation to risk in those who never worked on a plantation. All reported P values were based on 2-sided tests of significance.

RESULTS

The median age of the 7986 men at study enrollment (1965-1968) was 53 years (age range, 45-68 years). Median length of follow-up was 27 years. Range of follow-up was 1 month to 30 years. We identified 116 men who developed PD. Median age of diagnosis was 73.7 years (age range, 54-89 years). Median interval between baseline examination and PD onset was 17.5 years (range, 2-30 years).

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Table 1. Distribution of Years Worked on a Plantation According to Age at the Time of Study Enrollment

Age, y	Sample Size	Mean (SD) of Years Worked*	Range of Years Worked	Men Who Worked on a Plantation >10 y, %*
45-49	1829	2.5 (6.1)	0-34	6.3
50-54	2785	3.2 (7.4)	0-41	8.9
55-59	1590	3.6 (8.7)	0-47	10.9
60-68	1782	5.3 (11.2)	0-52	13.6
Overall	7986	3.6 (8.5)	0-52	9.8

*Values increased a significantly with age ($P<.001$).

Table 2. Average Age and Age-Adjusted Measures of Cigarette Smoking, Coffee Intake, and Exposure to Pesticides According to Number of Years Worked on a Plantation*

Variable	Reported No. of Years Worked on a Plantation (1965-1968) 0 (n = 5363)	1-10 (n = 1843)	11-20 (n = 315)	>20 (n = 465)
Age, y†	54.1 (5.4)	54.8 (6.0)	55.0 (5.3)	56.5 (6.0)
Pack-years of smoking	31.2 (29.9)	33.0 (29.8)	32.9 (28.7)	30.4 (28.4)
Current smoking status, %				
Past	24.5	27.2	23.3	27.8
Current	46.0	48.0	51.2	46.6
Coffee intake, oz/d	13.3 (12.9)	13.6 (12.2)	14.4 (12.0)	13.4 (11.5)
Pesticide exposure, y†‡	0.8 (2.8)	0.9 (2.9)	1.2 (3.1)	2.0 (5.1)

*Data are given as mean (SD) unless otherwise indicated.
†Values increased a significantly with increasing number of years worked on a plantation ($P<.001$).
‡Self-reported values collected at examinations received from the 1971-1974 period.

Table 3. Incidence of PD (Rate per 10 000 Person-years) According to Number of Years Worked on a Plantation and Years of Exposure to Pesticides

Duration, y	Sample Size	No. of PD Cases	Unadjusted	Age-Adjusted
Self-reported Plantation Work, 1965-1968				
0	5363	73	5.7	5.8
1-10	1843	24	5.5	5.4
11-20	315	7	9.6	9.2
>20	465	12	11.3*	10.3
Test for trend†	...	...	$P=.002$	$P=.01$
Overall	7986	116	6.1	...
Self-reported Exposure to Pesticides, 1971-1974				
0	3154	46	7.9	7.8
1	2663	33	6.5	6.5
2-3	587	9	8.1	8.2
>3	450	11	12.9	12.7
Test for trend†	...	...	$P=.08$	$P=.10$
Overall	6854	99	7.7	...

*Value indicates a significant excess risk of Parkinson disease (PD) compared with men who never worked on a plantation ($P=.02$).
†Test for trend is based on modeling the number of years worked on a plantation and the number of years of pesticide exposure as continuous variables.

Table 4. Estimated Relative Risk of Parkinson Disease in Men Who Worked on a Plantation Compared With Those Who Never Worked on a Plantation

Reported No. of Years Worked on a Plantation (1965-1968)	Estimated Relative Risk (95% Confidence Interval) Age-Adjusted	Risk Factor Adjusted*
0	Reference	Reference
1-10	0.9 (0.6-1.5)	1.0 (0.6-1.6)
11-20	1.6 (0.7-3.5)	1.7 (0.8-3.7)
>20	1.8 (1.0-3.3)	1.9† (1.0-3.5)
Test for trend‡	$P=.011$	$P=.006$

*Values were adjusted for age, pack-years of smoking, and coffee intake. Reference indicates all comparisons are made to the group with 0 years worked on a plantation.
†Value indicates significant excess of Parkinson disease compared with men who never worked on a plantation ($P=.046$).
‡Test for trend is based on modeling the number of years worked on a plantation as a continuous variable.

Table 1 lists the percentage of men who worked on a plantation for more than 10 years and mean duration of plantation work according to age at the time of study enrollment. In each instance, the duration and percentage of men who worked on a plantation for more than 10 years increased significantly with increasing age ($P<.001$). **Table 2** summarizes how other factors varied according to years worked on a plantation. There were no associations of smoking or coffee intake with plantation work. Pesticide exposure measured 6 years after study enrollment, however, increased consistently with years worked on a plantation ($P<.001$).

Table 3 gives observed incidence of PD according to the years worked on a plantation at the time of study enrollment and by the years of pesticide exposure reported 6 years later. After adjustment for age, the incidence of PD increased significantly with increasing years of plantation work ($P=.01$). The risk of developing PD nearly doubled in those who worked on a plantation for more than 20 years (10.30 per 10 000 person-years) compared with those who never worked on a plantation (5.80 per 10 000 person-years). Age-adjusted incidence of PD tended to increase with increasing years of exposure to pesticides ($P=.10$) although findings were not statistically significant. In this instance, reductions in sample size together with misclassification of pesticide exposure by recall may have limited statistical power.

Table 4 summarizes the excess risk of PD observed in plantation workers vs nonworkers after adjusting for potentially confounding effects of age, pack-years of smoking, and coffee intake. Compared with men who never worked on a plantation, the risk of PD was similar in those who worked from 1 to 10 years. The risk of PD in men who worked more than 20 years was nearly doubled compared with men who never worked on a plantation (relative risk [RR], 1.9; 95% CI, 1.0-3.5; $P=.046$). Although a threshold effect seemed to exist at more than 10 years of plantation work, a modest increase in risk con-

tinued to occur with further years of exposure ($P=.006$, test for trend).

As noted in the methods, data on exposure to either sugarcane or pineapple plantation work specifically was collected only at the later examination (1971-1974) and the intensity of exposure is less certain because part-time work was not differentiated from full-time work. Also, pineapple plantations employed fewer men (N=1243) than sugarcane plantations (N=2666). Based on 24-year follow-up, it appears that risk of PD increased with years of work on either type of plantation. For sugar plantation work, PD incidence increased from 6.6 per 10000 person-years in men who never worked on a plantation to 14.3 per 10000 person-years in men who worked more than 10 years (RR, 2.1; 95% CI, 0.9-5.1). For pineapple plantation work the risk of PD increased from 7.5 per 10000 person-years in men who never worked on a pineapple plantation to 11.0 in men who worked more than 10 years (RR, 1.4; 95% CI, 0.5-4.6).

COMMENT

Between 1885 and 1908, approximately 180000 Japanese workers migrated to the Hawaiian Islands to provide labor for sugar and pineapple plantations.[27] First- and second-generation Japanese American men enrolled in the Honolulu Heart Program were all born in the years 1900-1919 and those who worked on plantations would have done so between 1920 and 1985. Most (68%) of the men involved in plantation work were employed on sugarcane plantations. Case-control studies from China, Hong Kong, Taiwan, Canada, Sweden, and the United States suggest that agricultural work is associated with an increased risk of PD.[28-33] To our knowledge, this is the first prospective study demonstrating an association between agricultural work during midlife and the incidence of PD. Only relatively long-term work on a plantation (>10 years) was associated with this increase in risk.

While growing evidence implicates the neurotoxic effects caused by pesticide exposure as a possible factor in the pathogenesis of PD, it is important to emphasize that plantation workers experienced many other exposures unrelated to pesticides. Our data cannot discern which of these exposures may have influenced the development of the disease. The plantation environment was dusty and workers were highly exposed to all substances contained in dust including agrichemicals, metals, and soil pathogens. Interestingly, a common soil microorganism, *Nocardia asteroides*, has been found to cause selective nigral injury, with cytoplasmic inclusions resembling Lewy bodies and a movement disorder responsive to levodopa treatment in laboratory animals.[34-37] However, a case-control study in humans found no association between *N asteroides* serology and PD.[35] Exposures to metals such as copper, manganese, and combined exposures to lead-copper, lead-iron, and iron-copper, and manganese have been associated with increased risk of PD.[38] Notably, soil manganese content is known to be very high in Hawaii.[39]

Plantation living quarters were typically close making it possible that epidemics of pathogens selectively damaging dopaminergic neurons occurred similar to the worldwide epidemic of von Economo disease that caused postencephalitic parkinsonism.[40] A recent study found that persons with PD were more likely to work in either teaching or healthcare services than control subjects. Since these occupations are associated with high respiratory pathogen exposures, these findings were interpreted as consistent with an infectious cause of PD.[41] Recent work has examined the hypothesis that influenza A and other viruses may lead to formation of Lewy bodies and nigral cell death.[42]

The exposure most consistent with current theories of environmental causes of PD is agricultural chemicals. There are 2 commonly proposed pesticide exposure routes for farm workers. One is direct dermal and inhalation exposure and the other is consumption of contaminated well water. Four studies demonstrated a link between exposure to pesticides and PD.[29-32] In 3 of the 4, the entire relationship between agricultural work and PD was thought to be due to pesticide exposure after statistical adjustment for confounding data.

While not examining agricultural work directly, other studies of PD in rural settings have noted increased rates of PD associated with pesticide exposure[6,29,30,43-47] and consumption of well water.[8,11,48,49] Two studies have had negative results.[50,51] Interestingly, in the study by Tanner et al[51] conducted in China, no relationship between agricultural work and PD risk was found. Pesticides were not commonly used in Chinese farming at the time the study was conducted.

Although well water drinking was not directly assessed in our study, 92% of water used on the island of Oahu (and virtually all water used by plantations) during the time the cohort would have been working was well water according to the City and County of Honolulu Board of Water Supply. Since 1929, the Board of Water Supply Chemistry and Microbiology Laboratories have monitored the quality of Oahu's public water supply. Chemical agents including such metals as lead, mercury, and arsenic that could confound the observed association have not been detected.[52] However, it is possible that pesticides as field runoff could have contaminated wells on Hawaii's plantations.

Complete documentation of the historical use of pesticides in Hawaiian agriculture is unavailable. There are 2 reports that evaluate pesticide use in Hawaii during 1945-1970.[53,54] Pineapple growers used large amounts of insecticides and fumigants to control insect pests. Soil fumigants, primarily used to control nematodes in the pineapple industry, accounted for 60.7% (7364500 pounds) of all pesticides used in Hawaii, with 1,3-dichloropropene-1 and 1,2-dichloropane, 1,2-dibromoethane, and bromobenzylcyanide (Nemagon) the most common fumigants used. Synthetic insecticides came into common use between 1944 and 1964.[54] The insecticides most frequently used in the pineapple industry were the organochlorines dichlorodiphenyltrichloroethane (DDT), heptachlor, lindane, and chlordane and the organophosphates malathion and diazinon.[53]

Historically, biological controls have primarily been used to eradicate insect pests on sugarcane, and chemical insecticides have seldom been required. Herbicides

constituted more than 90% of the total amount of pesticides used on sugarcane and 32% of all pesticides used in Hawaii. Before 1945 only a few inorganic arsenic compounds were available to control weeds. Between 1945 and 1965 use of synthetic herbicides dramatically increased.[54] Commonly used herbicides during this period were pentachlorophenol, diuron, dalapon, sodium trichloroacetate, and the triazines—trazine and ametryn.[53]

The greatest application of insecticides in Hawaii was for termite control. Chlordane and DDT were the most commonly used organochlorine insecticides. Organochlorines were introduced into agricultural use immediately after World War II and pineapple plantations used these compounds extensively. During the latter years that cohort members would have worked on plantations (the 1960s and 1970s) organochlorines were replaced by organophosphorous insecticides malathion, diazinon, dibrom, parathion, dimethoate, and DDVP (2,2-dichlorovinyl dimethyl phosphate).

While the incidence of PD increased for those who were exposed to pesticides compared with unexposed individuals, this difference was not statistically significant. Sample size issues may play a role in these statistical findings because (1) data on pesticide use were unavailable in the full cohort of men, (2) follow-up for PD was shortened from 30 to 24 years, and (3) population incidence of PD is low. In addition, self-report of pesticide exposure is far less certain than data on plantation employment, since it depends on personal knowledge and recall of cumulative exposure episodes. Regular exposure to pesticides on plantations in Hawaii may have been more common than perceived by the worker, and many of those who reported not being exposed to pesticides could have had high levels of exposure. An effort is underway to identify specific work processes used when applying herbicides and insecticides on plantations in Hawaii during various periods.

Data presented herein together with recent reports of pesticide-induced animal models of parkinsonism[2,3] implicate occupational pesticide exposure as a likely factor responsible for increased incidence of PD in study subjects who had worked on plantations for more than a decade. Nevertheless, infectious agents or metals in soil and dust could also have contributed to the destruction of dopaminergic neurons.

Most pesticides that our subjects would have been exposed to are no longer used in the United States; however, they may still be used in other nations, especially in nations without rigorous regulatory agencies. Even if these substances are not in wide use, strong evidence implicating 1 or more could provide significant clues to the underlying cause of PD, and might facilitate recognition of potential neurotoxins to which persons may be exposed in industrial, military, or other agricultural circumstances. Continued investigation of specific herbicides and insecticides and application methods used during the years the cohort worked on plantations is ongoing, as is surveillance for additional PD cases. Analysis of these more specific and statistically powerful data may help strengthen the link between use of certain pesticides and risk of PD.

Accepted for publication June 6, 2002.

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Author contributions: *Study concept and design* (Drs Petrovitch, Ross, Abbott, Tanner, Blanchette, and White); *acquisition of data* (Drs Petrovitch, Ross, Masaki, Popper, White, and Mr Foley); *analysis and interpretation of data* (Drs Petrovitch, Ross, Abbott, Sanderson, Sharp, Tanner, Launer, and White); *drafting of the manuscript* (Drs Petrovitch, Ross, and Abbott); *critical revision of the manuscript for important intellectual content* (Drs Petrovitch, Ross, Sanderson, Sharp, Tanner, Masaki, Blanchette, Popper, Launer, White, and Mr Foley); *statistical expertise* (Drs Abbott, Sharp, and White); *obtained funding* (Drs Petrovitch, Ross, and White); *administrative, technical, and material support* (Drs Ross, Sanderson, Blanchette, Popper, White, and Mr Foley); *study supervision* (Drs Petrovitch, Ross, Masaki, Blanchette, and White).

This study was supported by grant DAMD17-98-1-8621 from the US Department of the Army, Washington, DC; contract NO1-AG-4-2149 from the National Institute on Aging, National Institutes of Health, Bethesda, Md; contract NO1-HC-05102 from the National Heart, Lung, and Blood Institute, Bethesda; Office of Research and Development Medical Research Service, Department of Veterans Affairs, Washington (Dr Ross); and contract HELD00B0059 from the National Institute for Occupational Health and Safety, Washington.

The information contained in this article does not necessarily reflect the position or the policy of the government, and no official endorsement should be inferred.

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APPENDIX E

Frequency of bowel movements and the future risk of Parkinson's disease

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Article abstract—*Background:* Constipation is frequent in PD, although its onset in relation to clinical PD has not been well described. Demonstration that constipation can precede clinical PD could provide important clues to understanding disease progression and etiology. The purpose of this report is to examine the association between the frequency of bowel movements and the future risk of PD. *Methods:* Information on the frequency of bowel movements was collected from 1971 to 1974 in 6790 men aged 51 to 75 years without PD in the Honolulu Heart Program. Follow-up for incident PD occurred over a 24-year period. *Results:* Ninety-six men developed PD an average of 12 years into follow-up. Age-adjusted incidence declined consistently from 18.9/10,000 person-years in men with <1 bowel movement/day to 3.8/10,000 person-years in those with >2/day ($p = 0.005$). After adjustment for age, pack-years of cigarette smoking, coffee consumption, laxative use, jogging, and the intake of fruits, vegetables, and grains, men with <1 bowel movement/day had a 2.7-fold excess risk of PD versus men with 1/day (95% CI: 1.3, 5.5; $p = 0.007$). The risk of PD in men with <1 bowel movement/day increased to a 4.1-fold excess when compared with men with 2/day (95% CI: 1.7, 9.6; $p = 0.001$) and to a 4.5-fold excess versus men with >2/day (95% CI: 1.2, 16.9; $p = 0.025$). *Conclusions:* Findings indicate that infrequent bowel movements are associated with an elevated risk of future PD. Further study is needed to determine whether constipation is part of early PD processes or is a marker of susceptibility or environmental factors that may cause PD.

NEUROLOGY 2001;57:456–462

Constipation is frequent in patients with PD.[1-17] Case reviews further suggest that constipation can precede the extrapyramidal symptoms of clinical PD by many years.[1,2] Such reviews, however, are subject to uncertain recall of constipation histories and to confounding due to episodes of constipation that can occur naturally with advancing age.

Clear demonstration that constipation can precede clinical PD is important because it suggests that recognition of pathogenic mechanisms in the PD process could occur before the emergence of motor symptomatology. Identification of constipation as a risk factor for PD could also help identify early or suspected disease and provide for opportunities to develop or investigate intervention strategies. Unfortunately, there are no prospective follow-up studies that confirm that constipation can precede the clinical manifestations of PD. The purpose of this report is to examine the association between the frequency of bowel movements and the future risk of PD based on 24 years of follow-up of a cohort of asymptomatic men enrolled in the Honolulu Heart Program.

Materials and methods. *Study sample.* From 1965 to 1968, the Honolulu Heart Program began following 8006 men of Japanese ancestry living on the island of Oahu, HI, for the development of cardiovascular disease.[18-20] At the time of study enrollment, subjects were aged 45 to 68 years. Initial screening consisted of a baseline physical examination and documentation of cardiac and neurologic conditions to identify prevalent cases of cardiovascular disease. Procedures were in accordance with institutional guidelines and approved by an institutional review committee. Informed consent was obtained from the study participants.

The Honolulu Heart Program is now in its 36th year of follow-up. During this period, surviving members of the original cohort participated in repeat examinations and were tracked for morbidity and mortality outcomes through a comprehensive system of surveillance that included a review of hospital discharges, death certificates, and autopsy records. As of 1990, less than 1% of the original cohort had moved off the island of Oahu resulting in an out-migration rate of about one per thousand per year. Validity studies have indicated that nearly 100% of hospital discharge events have been identified.

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Supported by a grant from the US Department of the Army (DAMD17-98-1-8621), by the NIH (National Institute on Aging Contract N01-AG-4-2149 and grant 1 R01 AG17155-01A1, National Heart, Lung, and Blood Institute Contract N01-HC-05102, a Research Centers in Minority Institutions Award P20 RR 11091, and a National Institute of Environmental Health Sciences grant 1 R01 NS41265-01), and by Department of Veterans Affairs Medical Research funds.

The information contained in this article does not necessarily reflect the position or the policy of the government, and no official endorsement should be inferred.

Received January 19, 2001. Accepted in final form February 7, 2001.

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For this report, follow-up for incident PD began at a repeat examination that occurred from 1971 to 1974 when information on the frequency of bowel movements was first collected. Subjects examined included 6860 men, approximately 90% of the surviving members of the original cohort. After exclusion of 64 men with missing bowel movement data and six men with prevalent PD, 6790 men remained for follow-up.

Frequency of bowel movements and confounding information. At the time when follow-up began (1971 to 1974), study participants were asked about their usual daily bowel movement frequency and categorized as having <1, 1, 2, and >2 bowel movements/day. Information on the use of laxatives was also collected. Other confounding information collected at the beginning of follow-up and known to be related to PD included age, pack-years of cigarette smoking, and intake of coffee.[21,22] Participants were also asked about jogging and intake of fruits, vegetables, and grains. Men were defined to be joggers if they reported that they jogged or ran intermittently or regularly without further characterization in terms of distance and intensity. While other measures of physical activity were not available when follow-up began (1971 to 1974), a physical activity index (an overall measure of 24-hour metabolic output) that was measured at the time of study enrollment (1965 to 1968) was also assessed.[23] Measurement of food and coffee intake was based on a food frequency questionnaire in which subjects were asked about consumption of these items during the previous week.[21]

PD case finding and diagnosis. For this report, 24 years of follow-up data are available on incident PD after collection of information on bowel movement frequency (1971 to 1974). Prior to 1991, cases of PD were identified through a review of all hospital records of cohort members for new and preexisting diagnoses of PD, an ongoing review of all Hawaii death certificates, and a review of medical records at the offices of local neurologists for all cohort members with PD identified within the previous 25 years.

Beginning in 1991, the diagnosis of PD was based on a complete screening of the participating cohort at examinations that occurred from 1991 to 1993 and again from 1994 to 1996. All subjects were questioned about diagnoses of PD, symptoms of parkinsonism (any two of rest tremor, bradykinesia, rigidity, or postural instability), and PD medications by a structured interview. Those with a history or sign of parkinsonism were referred to a study neurologist who administered standardized questions about symptoms and onset of parkinsonism, previous diagnoses, and medication use, followed by a comprehensive and standardized neurologic examination. A diagnosis of PD was based on consensus among the study neurologists according to published criteria.[24] These required that the subject have the following: 1) parkinsonism; 2) a progressive disorder; 3) any two of a marked response to levodopa, asymmetry of signs, asymmetry at onset, or initial onset tremor; and 4) absence of any etiology known to cause similar features. Cases of parkinsonism related to progressive supranuclear palsy, multisystem atrophy, cerebrovascular disease, drug-induced parkinsonism, post-encephalitic parkinsonism, or post-traumatic parkinsonism were not included among the cases of PD. Further description of PD case finding and diagnosis is described elsewhere.[21,25]

Statistical methods. Crude and age-adjusted incidence rates of PD in person-years were estimated according to bowel movement frequency based on the 24 years of follow-up available in the 6790 men who were examined from 1971 to 1974.[26] Age-adjusted risk factors across levels of bowel movement frequency were also derived.[26] To test for an independent effect of bowel movement frequency on PD after adjusting for age and the other covariates, proportional hazards regression models were used.[27] In this instance age, coffee intake, pack-years of cigarette smoking, and combined intake of fruits, vegetables, and grains were modeled as continuous variables, while jogging and laxative use were modeled as dichotomous variables (yes versus no). While frequency of bowel movements was modeled as a continuous risk factor, relative risks of PD (and associated confidence intervals) were also estimated comparing the risk of PD for men with <1 bowel movement/day to men with 1, 2, and >2/day. All reported p values were based on two-sided tests of significance.

Results. The average age of the 6790 men was 60 years (range: 51 to 75) at the time when questions were asked about usual bowel movement frequency (1971 to 1974). Over the 24-year course of follow-up, 96 men developed PD. The average age at the time of diagnosis was 73 years (range: 55 to 90), and the average time to diagnosis was 12 years (range: 2 months to 24 years).

Table 1 shows the percent of men with <1, 1, 2, and >2 bowel movements/day and the use of laxatives according to age when follow-up began. Approximately two-thirds of the men reported having 1 bowel movement/day while a quarter reported having 2/day. Overall, 4.3% of the men had <1 bowel movement/day and 6.3% had >2/day. The percent of men with infrequent bowel movements (<1/day) rose from 3.6% in men aged 51 to 55 years to 9.1% of men aged 71 to 75 ($p < 0.001$) whereas the percent of men with >2/day declined from 6.8 to 3.6% ($p = 0.015$). Although associations appear modest, the percent of men with 1 bowel movement/day also increased with age whereas the percent of men with 2/day declined. Along with the increase in infrequent bowel movements with advancing age (<1/day), there was also an increase in the reported use of laxatives. Laxative use increased from 16.4% in the youngest group of men to 29.6% in those who were the oldest ($p < 0.001$).

Table 2 describes the way in which age and the age-adjusted covariates varied according to bowel movement frequency. Age, coffee intake, and use of laxatives declined with increasing number of bowel movements/day ($p < 0.001$). Daily consumption of coffee in men with <1 bowel movement/day was (on average) 3.4 oz more than in men with >2/day (14.0 versus 10.6 oz/day). The percent of men who used laxatives was more than doubled in men with infrequent bowel movements (<1/day) as compared with men who had >2/day (44.7 versus 18.0%). Pack-years of smoking appeared to increase with frequency of bowel movements ($p = 0.033$), although there was no relation with the percent of men who were current and past cigarette smokers. Although jogging was not significantly related to bowel movement frequency, the percent of men who jogged was nearly doubled in men with >2 bowel movements/day (11.1%) versus men with <1/day (5.9%). Intake of fruits, vegetables, and grains increased significantly but modestly with increasing bowel movement frequency.

Table 1 *Percent of men with <1, 1, 2, and >2 bowel movements/day and percent of men who used laxatives according to age at the beginning of study follow-up (1971 to 1974)*

Age	Sample size	Bowel movements/d				Laxative use (1402)
		<1 (289)*	1 (4371)	2 (1704)	>2 (426)	
51–55	1642	3.6	61.7	27.9	6.8	16.4
56–60	2421	3.8	63.4	26.2	6.6	19.3
61–65	1353	3.4	65.7	23.9	7.0	21.2
66–70	1011	5.7	69.0	20.6	4.7	26.8
71–75	363	9.1	65.0	22.3	3.6	29.6
Test for trend	—	$p < 0.001$†	$p < 0.001$†	$p < 0.001$‡	$p = 0.015$‡	$p < 0.001$†
Overall	6790	4.3	64.4	25.1	6.3	20.6

* Sample size.
† Significant increase with age.
‡ Significant decrease with age.

The incidence of PD according to frequency of bowel movements is shown in table 3. Both unadjusted and age-adjusted incidence increased consistently with decreasing bowel movement frequency. The age-adjusted incidence of PD increased from 3.9/10,000 person-years in men with >2 bowel movements/day to 18.9/10,000 person-years in men with <1/day ($p = 0.005$). Although modest, the average age at PD diagnosis declined consistently with decreasing bowel movement frequency. Men with infrequent bowel movements (<1/day) had a diagnosis of PD that was an average of 18 months sooner than those with >2 bowel movements/day. This latter association was not significant.

Table 4 further describes the estimated relative risk of PD in men with <1 bowel movement/day versus men whose bowel movements were more frequent. After adjustment for age and the other covariates, men with <1 bowel movement/day had a 2.7-fold excess risk of PD versus men with 1/day (95% CI: 1.3, 5.5; $p = 0.007$). The risk of PD in men with <1 bowel movement/day increased to a 4.1-fold excess when compared with men with 2/day (95% CI: 1.7, 9.6; $p = 0.001$) and to a 4.5-fold excess versus men with >2/day (95% CI: 1.2, 16.9; $p = 0.025$).

Although data may be too limited to carefully assess time-varying effects, the association between the frequency of bowel movements and the risk of PD appeared similar for diagnoses made early versus later into follow-up. As compared with men with ≥1 bowel movement/day, men whose bowel movements were less frequent had a 2.9-fold excess risk of PD in the first 12 years of follow-up (95% CI: 1.1, 7.6; $p = 0.030$) and a similar 3-fold excess for diagnoses that were made in the 12-year period that followed (95% CI: 1.0, 8.6; $p = 0.042$).

Discussion. Recall bias is a major weakness of case-control studies in describing an association between constipation and future risk of clinical PD.[1,2]

Table 2 *Average age and age-adjusted covariates according to bowel movement frequency at the beginning of study follow-up (1971 to 1974)*

Covariate	Bowel movements/d			
	<1 (289)*	1 (4371)	2 (1704)	>2 (426)
Age¶	61.5 ± 6.3†	60.3 ± 5.5	59.6 ± 5.4	59.5 ± 5.0
Coffee intake, oz/d¶	14.0 ± 11.6	12.9 ± 11.1	12.0 ± 11.8	10.6 ± 9.3
Pack-years of smoking‡	34.4 ± 35.5	34.2 ± 32.5	34.5 ± 32.8	39.1 ± 35.5
Current smoking status				
Past, %	32.4	37.3	36.9	32.0
Current, %	36.3	34.3	32.9	39.5
Laxative use, %¶	44.7	20.7	17.0	18.0
Jogging, %	5.9	9.1	8.8	11.1
Intake of fruits, vegetables, and grains, g/d§	438 ± 246	432 ± 242	443 ± 250	468 ± 303

* Sample size.
† Mean ± standard deviation.
‡ Significant increase with increasing bowel movement frequency ($p = 0.033$).
§ Significant increase with increasing bowel movement frequency ($p = 0.011$).
¶ Significant decrease with increasing bowel movement frequency ($p < 0.001$).

Table 3 *Incidence of PD according to frequency of bowel movements*

Bowel movements/d	Sample size	Incident PD cases	Incidence, rate/10,000 person-years	
			Unadjusted	Age-adjusted
<1	289	10	19.6	18.9
1	4371	66	8.0	7.9
2	1704	17	5.2	5.4
>2	426	3	3.8	3.9
Test for trend	—	—	$p = 0.002$	$p = 0.005$
Overall	6790	96	7.5	—

Patients with PD may be prone to errors in reporting of past symptoms because of uncertain recall of constipation histories that may have predated a diagnosis of PD by many years.

The major strength of this report is that data are from individuals who were asked about usual bowel movement frequency an average of 12 years prior to the development of PD. Bias that might have been introduced through the use of medications for PD is also absent. Although constipation has always been known to coexist in patients with PD, this is the first large-scale prospective study to show a significant association between infrequent bowel movements and an elevated risk of PD in later life. In addition, the risk of PD increased consistently as frequency of bowel movements decreased. Although not significant, data further suggest that infrequent bowel movements (<1/day) are also associated with an earlier age at onset of PD. Among the men with PD, a diagnosis was made before the age of 60 years in two of the 10 men (20%) with <1 bowel movement/day, six of the 66 men (9.1%) with 1/day, and in none of the 20 men with ≥2/day. Infrequent bowel movements also appeared to be associated with an elevated risk of PD for diagnoses made early and late into the 24-year follow-up considered in this report. Whether these findings can be duplicated in other prospective studies and extended to include women and other ethnic groups warrants further study.

Table 4 *Estimated relative risk of PD in men with <1 bowel movement/day versus men whose bowel movements were more frequent*

Adjustment	Risk of PD in men with <1 bowel movement/d as compared with men with 1, 2, and >2/d		
	1/d	2/d	>2/d
Age-adjusted	2.3‡	3.4§	4.8¶
	(1.2, 4.5)†	(1.6, 7.5)	(1.3, 17.3)
Risk factor adjusted*	2.7‖	4.1**	4.5††
	(1.3, 5.5)	(1.7, 9.6)	(1.2, 16.9)

* Adjusted for age, pack-years of cigarette smoking, coffee consumption, laxative use, jogging, and intake of fruits, vegetables, and grains.
† 95% confidence interval.
‡ Excess of PD versus men with 1 bowel movement/d ($p = 0.013$).
§ Excess of PD versus men with 2 bowel movements/d ($p = 0.002$).
¶ Excess of PD versus men with >2 bowel movements/d ($p = 0.018$).
‖ Excess of PD versus men with 1 bowel movement/d ($p = 0.007$).
** Excess of PD versus men with 2 bowel movements/d ($p = 0.001$).
†† Excess of PD versus men with >2 bowel movements/d ($p = 0.025$).

Although there exists the possibility of diagnostic misclassification among the PD cases, with some having multiple-system atrophy rather than PD, the number of such instances is likely to be small.[28] In the current report, the chance of a diagnosis of an atypical parkinsonism syndrome is further reduced by consensus agreement on the presence of any two of a marked response to levodopa, asymmetry of signs, asymmetry at onset, or initial onset tremor, signs generally thought to be more specific for PD. Incidence of PD in the Honolulu Heart Program is also in general agreement with rates observed in Europe and the United States.[25,29] In addition, among the men with PD, 10 cases had an autopsy. Seven cases were confirmed by the presence of Lewy-bodies in the substantia nigra, while pathologic examination of the remaining three has not been completed.

Although bowel movement and constipation questionnaires vary among study samples, frequency of bowel movements and use of laxatives in the men in the Honolulu Heart Program are also similar to frequencies described elsewhere.[30-35] In the National Health Interview Survey on Digestive Diseases, 64% reported having 7 to 13 bowel movements/week and 11.7% reported having 14 to 20/week.[30] Although use of laxatives in this cohort was less than in the Honolulu Heart Program, it was not markedly less (increasing from 7.4% in men aged 50 to 59 years to 19.3% in men aged 70 to 79). In the National Health and Nutrition Examination Survey, 64 to 74% recorded daily defecation.[31] In an industrial community, 5.1% reported having <5 bowel movements/week, 68% reported having 5 to 7/week, and 26% reported having 2/day.[32] The latter corresponds well with the 25.1% of men in the current cohort who reported having 2 bowel movements/day, although laxative use in this industrial community was high (27.9% in subjects aged 50 to 59 years to 50% in those who were older). In one report in which bowel movement frequency was recorded in the same manner as in the current sample, 58.9% reported having 1 bowel movement/day, approximately 30% had 2/day, with the remaining sample being evenly divided between those with <1 and >2/day.[33] Use of laxatives was also reported by 18.5% of the sample, similar to the Honolulu cohort.

As might be expected, men in the Honolulu Heart Program also reported using a variety of different types of laxatives. Preference for a specific laxative, however,

did not seem to vary greatly by frequency of bowel movements or among cases and non-cases of PD. Among those who used laxatives, approximately 25% were taking milk of magnesia, citrate of magnesia, or magnesium sulfate. Over-the-counter stimulants were used by 12.1% of laxative users whereas 9.2% used prunes, 7.1% used enemas or suppositories, 5.5% used bulk-forming laxatives, and 2.5% used lubricants. Rarely used laxatives included an assortment of fruits, vegetables, cereals, tea, and coffee. Use of laxatives was not associated with the risk of PD.

Although constipation is the most common gastrointestinal disorder among patients with PD, careful comparisons with matched controls are few and equivocal. Variation among reported rates is also high. In four case-control studies, prevalence of constipation ranged from 28 to 61% in patients with PD as compared with 6 to 33% in controls.[3-6] Others report that constipation or prolonged transit time can afflict up to 80% of patients with PD.[7] Among studies suggesting that constipation can precede PD, one source reported that in a series of 178 patients with PD, 46% had constipation prior to the first symptoms of PD, whereas in spouse controls (largely women), 28% had complaints of constipation.[1] In another study, constipation was reported to have occurred prior to a diagnosis of PD in 10 of 12 patients by an average of 16 years.[2] In the Honolulu cohort, among the men with infrequent bowel movements (<1/day) who later developed PD, onset occurred an average of 10 years into follow-up (range: 5 months to 19 years).

Despite a history of documentation of an association between constipation and PD since first described by James Parkinson in 1817,[36] pathologic mechanisms relating constipation and PD remain poorly understood. Increased colonic transit time may be a manifestation of the same processes that cause the motor symptoms of PD. Evidence supporting this includes the findings of depletion of dopamine-producing neurons in the colon and the presence of Lewy-bodies in the myenteric plexus.[8,9] Importantly, delayed colonic transport in PD has been found to be independent of age, physical activity, and medication.[10] Additionally, CNS derangements may lead to abnormalities in skeletal muscle of the pelvic floor and anal sphincter that control defecation. Evidence for this includes radiologic studies in patients with PD demonstrating paradoxical anal sphincter muscle contraction during defecation and anorectal manometry showing hypercontractility of the external sphincter.[4,8,11,12] As a result, it appears that both autonomic and CNS abnormalities contribute to constipation in PD, and it is possible that these changes may be prodromal symptoms of the impending extrapyramidal syndrome.

Effects of diet and physical activity on gastrointestinal symptomatology and PD may also exist, although none has been clearly identified.[8,13,14] In one report based on 19 patients with PD, dietary intake of insoluble fiber was associated with improvements in constipation, extrapyramidal function, and bioavailability of levodopa.[15] In the current study, adjustments for jogging and intake of fruits, vegetables, and grains had no effect on the association between bowel movement frequency and the risk of PD. Although not measured when the frequency of bowel movements was first assessed, adjustment for the overall physical activity index[23] that was measured at the time of study enrollment (1965 to 1967) also failed to alter the observed relation between bowel movement frequency and PD in the Honolulu sample.

As noted by others and confirmed here, defecatory dysfunction can precede the clinical diagnosis of PD, suggesting that constipation could be one of the earliest features of PD processes that predate motor symptomatology by an average of 10 years or longer.[2] Defecatory dysfunction is also thought to be associated with severity and duration of PD,[8] although such a relation has not been confirmed.[16] It has further been suggested that frequent and severe constipation that is resistant to therapy could be a symptom of the early signs of PD, although a careful distinction must be made from constipation that occurs naturally with advancing age.[1,6,7]

Failure for constipation to be relieved by laxatives could be a marker of autonomic dysfunction that precedes PD pathology, or it could be a sign that PD processes have already begun. Although data in the current report are too limited to examine constipation that is not relieved by laxatives, the risk of PD appeared highest (26.6/10,000 person-years) in the cohort of men who reported using laxatives and continued to have <1 bowel movement/day. Insufficient data also prevent a clear assessment of interaction effects between frequency of bowel movements and use of laxatives.

It may also be that bowel movement frequency in the elderly has a weaker association with future PD compared with those who are younger, simply because infrequent bowel movements in the elderly become common relative to the incidence of PD. Unfortunately, repeat measurement of bowel movement frequency in the Honolulu cohort did not occur until late into the 24-year follow-up (1991 to 1993). Although bowel movement frequency declined over this period (simply because of the effects of age), bowel movement frequencies reported at the baseline and the later examination were positively related ($p < 0.001$). Using data from the later examination (1991 to 1993), future PD continued to be elevated in men with <1 bowel movement/day. In 3,397 men (aged 71 to 93 years) without PD in whom repeated bowel movement data were available, incident PD was observed in nine men. Among those who reported having <1 bowel movement/day, 1% developed PD (2/223) whereas 0.2% (7/3174) developed PD in those whose bowel movements were more frequent. Although far from conclusive based on the small number of cases, additional follow-up of this sample is expected to improve the opportunity to

more carefully assess the association between bowel movements and the future risk of PD in this elderly sample of men. In addition, the effects of infrequent bowel movements (<1/day) that may have appeared at the baseline examination (1971 to 1974) and persisted until the later examination (1991 to 1993) can also be examined.

In terms of clinical implications in the elderly, demonstration that the association between bowel movement frequency and the risk of PD weakens with advancing age means that information on bowel movement frequency must be measured as early in life as possible. The use of more comprehensive instruments for the collection of constipation histories may also be warranted. Although clinical implications must be defined, combining information on constipation with other factors, such as a positive family history and other motor deficits, could have some uses for identifying high-risk individuals for future PD. It may be worthwhile to document constipation histories from the suspected appearance of PD to its clear clinical presence. Combining constipation that is resistant to therapy with other factors could also provide a means for broadening enrollment into neuroprotective trials by including high-risk groups of asymptomatic individuals with early motor symptomatology.

In light of the observed findings from the Honolulu Heart Program and elsewhere,[1-17] it remains to be determined whether constipation is related to the underlying pathophysiologic processes in PD development, is a sign of early PD, or is a disease marker linked to other susceptibility or environmental factors. Identifying constipation as a risk factor for PD could lead to more effective strategies for identifying early or suspected disease and could provide for opportunities for prevention and intervention.

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Time trends in the incidence of parkinsonism in Olmsted County, Minnesota

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Article abstract—*Objective:* To investigate time trends in the incidence of parkinsonism and PD over a 15-year period (1976 to 1990). *Methods:* The authors used the medical records–linkage system of the Rochester Epidemiology Project to identify incidence cases of parkinsonism in Olmsted County, MN, over three 5-year periods, 1976 to 1980, 1981 to 1985, and 1986 to 1990. PD and other types of parkinsonism were classified using defined criteria. Population denominators were derived from census data and were corrected by removing prevalent cases of parkinsonism. *Results:* Over the 15 years of the study, 364 cases of parkinsonism were identified; 154 (42%) of them had PD. The incidence of parkinsonism remained stable over the three 5-year periods for the age classes 0 to 39, 40 to 59, and 60 to 69 years. For the age class 70 to 99 years, there was some increase over time mainly owing to an increased incidence of drug-induced parkinsonism. The incidence of PD remained stable over the three 5-year periods for all age classes. Results were similar when considering men and women separately. No birth-cohort effect was present for parkinsonism. Comparison with three previous studies in the same population did not reveal any major long-term secular trends in the incidence of parkinsonism. *Conclusions:* The findings for PD over 15 years and comparison of the findings with historical data for parkinsonism over half a century suggest that no major environmental risk factors for PD (e.g., environmental toxins, drugs, diet constituents, or infectious agents) were introduced or removed from this population during these periods.

NEUROLOGY 2001;57:462–467

The independent role of susceptibility genes and environmental risk factors and their interactions in the etiology of PD remain controversial.[1-3] Time trends in the incidence of PD may contribute to generating new etiologic hypotheses or may serve as a reference against which to test etiologic hypotheses based on laboratory findings (e.g., the recent suggestion that the pesticide rotenone could be an environmental cause of PD).[4] In addition, time trends in the incidence of PD and parkinsonism have public health uses for projecting the future burden of these disabling conditions and for planning medical services. Unfortunately, data on time trends are limited because it is difficult to measure the incidence of PD over time in a defined population. The limited current data are derived from counts of existing diagnoses obtained through medical record review and physician surveys or from a records-linkage system.[5-9]

We investigated time trends in the incidence of parkinsonism and PD over three 5-year periods (quinquennia) in Olmsted County, MN. In addition, we explored long-term trends in the incidence of parkinsonism by comparing our findings with those from three previous studies in the same population.[7-9] This study was made possible by the records-linkage system serving this community and was part of a broader project partly described elsewhere.[10,11]

Methods. *Case ascertainment.* We ascertained cases of parkinsonism through the records-linkage system of the Rochester Epidemiology Project, which provides the infrastructure for indexing and linking essentially all medical information of the population of Olmsted County, MN.[12,13] Each provider in the community employs a dossier system (or unit record) whereby all medical information for each individual is accumulated in a single record. Medical diag-

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Supported by an NIH grant (NS33978) and the Rochester Epidemiology Project (AR30582).

Presented in part at the 50th Annual Meeting of the American Academy of Neurology; Minneapolis, MN; April 1998.

Received December 4, 2000. Accepted in final form March 27, 2001.

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APPENDIX F

Longer QT Interval in Midlife Predicts Subsequent Development of Parkinson's disease: The Honolulu-Asia Aging Study

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Abstract

Background: The QT interval, an electrocardiographic indicator of heart cell repolarization, tends to lengthen with autonomic dysfunction and with aging. Prolongation of the QT segment has been reported in patients with Parkinson's disease (PD) and Lewy bodies have been found in synpathetic neurons in the heart in PD. The longitudinal design of the Honolulu Heart Program and the Honolulu-Asia Aging study provides opportunity to examine possible relationships between the QT interval measured in midlife and the subsequent development of Parkinson's disease.

Methods: 8004 Japanese-American men aged 45-65 received ECGs in 1965, 1967, and 1971. Autopsy information was available for 300 participants who died 1991-2000. PD incidence over approximately 30 years of observation was compared among 4 subgroups defined according to 1965 rate-corrected QT intervals: (group 1) <392 msec (lowest quartile of QT length), (group 2) 392-424 msec (25^{th} to 75^{th} percentile), (group 3) 425-439 msec (between the 75^{th} and 90^{th} percentile), and (group 4) >=440 msec (10% with longest QT). Risk for developing PD was estimated by proportional hazards models, controlling for age, cigarette smoking, and caffeine intake. Similar analyses were conducted using a composite indicator of QT length based on ECGs in 1967 and 1971. Correlations between the QT interval and Lewy bodies in the brain were examined for 300 autopsied subjects.

Results: Relative risk of PD increased with increasing QT interval as follows: Group 1 was the reference group; group 2 -- RR=1.44 (95% CI= 0.93-2.25); Group 3 -- RR=1.24 (95% CI=0.68-2.28); group 4 -- RR=2.19 (95% CI=1.21-3.99, p=.01). Median duration between QT measurement and diagnosis of PD was 19 years. The association was statistically significant only for cases developing before age 75, and was strongest when the disease was diagnosed more than

19 years after QT measurement. Correlation of PD incidence with a composite 1967/1971 indicator of QT length was consistent but of borderline statistical significance. Baseline QT interval was marginally associated with Lewy bodies in the pigmented brainstem nuclei at death. Conclusions: Among Japanese-American men, QT interval length in midlife predicted both clinical and neuropathologic PD endpoints. The association with clinical PD was limited to cases with onset before age 75 but was not a harbinger of imminent clinical disease. Risk seemed to increase continuously between the shortest and longest intervals, and was not limited to persons with the long QT syndrome. We speculate that the association reflects a constitutional predisposition to the development of Lewy bodies and to PD appearing before age 75.

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We speculate that some PD cases occurred in men who were predisposed by virtue of an inherited or acquired condition indicated by a prolonged QT interval in midlife. Such a condition could produce reduced cardiac sympathetic innervation well before signs and symptoms of PD became apparent. Alternatively, ion transport processes involved with prompt reolarization in heart cells may be related to molecular mechanisms essential to function and survival of dopaminergic neurons in the substantia nigra. ////////////////////////////////////

INTRODUCTION

The QT interval, determined from electrocardiography (ECG), is that segment of the heart's action potential (??) cycle during which repolarization of the cell membrane occurs.{XXXXXXXXXXXXXX} Its duration reflects calcium, potassium, and sodium ion pumping and is influenced by heart rate, autonomic inervation, a variety of drugs, a number of genetic conditions, and other factors including aging.{xxxxxxxxxxxxxxx} Reports from Japan have noted prolongation of the QT interval in patients with Parkinson's disease (PD). {ISHIZAKI1996, OKA1997} A recent Italian study showed QT prolongation in early, recently recognized PD cases. {QUADRI2000} A very recent autopsy study demonstrated Lewy bodies in sympathetic neurons in the heart, as well as in the brain of patients with Parkinson's disease.{xxxxxxxxxxxxxxxxxxxxxxxxxx}

An increase in QT interval is consistent with the generalized autonomic disturbance that commonly occurs in PD. {MARTIGNONI1986, TURKKA1987, TAKAHASHI1991, IEDA1999} In patients with clinically apparent disease it could be the result of the same neural and neurotransmitter metabolism abnormalitites as responsible for the more obvious signs and symptoms of Parkinson's disease. If present before the disease is recognized it could reflect either a constitutional susceptibility to the development of the disease, or an early, autonomic manifestation, preceeding motor signs. The analyses reported here address the relationship between the QT interval measured in midlife with subsequent development of PD in Japanese-American men participating in the longitudinal Honolulu Heart Program and the Honolulu-Asia Aging Study.

PATIENTS AND METHODS

The Honolulu Heart Program was established in 1965 with the examination of 8,006 men of Japanese ancestry 45-68 years old and living on the island of Oahu, Hawaii. The initial examination consisted of face-to-face interviews and physical evaluation. Demographic, dietary, and health status data were obtained. {YANO1984, HEILBRUN1985} The study is now in its 36th year of follow-up with continued surveillance of hospitalization and death records. Follow-up examinations were performed from 1968 to 1971, 1971 to 1974, 1991 to 1993, and 1994 to 1996. Research on neurodegenerative diseases of aging began in 1991 with establishment of the Honolulu-Asia Aging Study. Procedures were approved by an institutional review committee and informed consent was obtained from all participants. Details regarding study design have been previously published. {GRANDINETTI1994, WORTH1970, WHITE1996}

PD Case Finding and Diagnosis

For this report, 30 years of follow-up data were available. Incident cases of PD were identified through four sources. {MORENS1996} Prior to 1991, the sources were: 1) review of all cohort member's hospitalization records for all diagnoses of PD, 2) ongoing review of all Hawaii death certificates, and 3) review of medical records of all patients with PD from the offices of local neurologists cross checked with the cohort member list. {GRANDINETTI1994, MORENS1996}

After 1991, the diagnosis of PD was based on complete re-examinations of the entire cohort from 1991 to 1993 and 1994 to 1996. During the 1991 to 1993 examination, {MORENS1996} all subjects were questioned about history of PD, symptoms of parkinsonism (tremor, bradykinesia, rigidity, or postural instability), and PD medications by structured interview. Research technicians were trained to recognize clinical signs of parkinsonism including gait disturbance, tremor, or bradykinesia. Subjects with a history of PD or

parkinsonism symptoms or signs were referred to a study neurologist who administered standardized questions about symptoms and onset of parkinsonism, previous diagnoses, and medication usage, followed by a comprehensive and standardized neurological examination that included the Unified Parkinson's Disease Rating Scale. {LANG1989} Diagnosis of PD was based on consensus from two neurologists according to published criteria.{WARD1990} These required that the subject have:1) parkinsonism, 2) a progressive disorder, 3) any two of marked response to levodopa, asymmetry of signs, asymmetry at onset, or initial onset tremor, and 4) absence of any etiology known to cause similar features. Cases of parkinsonism related to other neurodegenerative disorders, cerebrovascular disease, medications, trauma, or post encephalitic parkinsonism were not included among cases of PD. Additional cases of PD were identified during the 1994 to 1996 examination through structured interviews inquiring about history of PD or PD medications. These cases were confirmed by a study neurologist through record review and application of the criteria above.

Age at diagnosis was used instead of age at onset to avoid inaccuracies associated with recall of symptom onset for a chronic disease with gradual onset. At study enrollment there were two prevalent cases of PD excluded from this analysis.

Assessment of ECG QT Interval

An ECG was performed on 8004 Japanese-American men aged 45-65 on four occassions: at baseline (1965-67), second exam (1967-69, third exam (1971-74), and fourth exam (1961-63). Specific methods varied slightly at the different examinations, but were consistent with at any specific examination. All analyses were done after correction for heart rate (determined from the ECG) using the standard correction XXXXXXXXXXXXXXXX.

Statistical Analysis

Most analyses addressed risk for developing PD estimated by proportional hazards models, controlling for age, usual cigarette smoking, pack years of cigarette smoking and caffeine intake during the 24 hours prior to the examination (estimated from dietary information).

RESULTS

Median age of the 7,986 men for whom QT intervals were measured at study enrollment (1965-1968) was 53 years (range: 45-68). Median length of follow-up was 27 years. Minimum follow-up was 1 month to the first death and maximum follow-up was 30 years. Among the men, 137 developed PD over the three decades of follow-up. Median age at diagnosis was 73.7 years (range: 54-89).

The relationship between the pulse rate-corrected QT interval measured in electrocardiographic recordings at the 1965-68 examination and the subsequent development of PD was examined in Cox proportional hazards multivariate analyses. Age, caffeine consumption in the prior 24 hours, usual number of cigarettes smoked per day, and accrued pack years of cigarette smoking were taken into account as covariates. Controlling for these factors, the QT interval (as a continuous variable) was significantly associated with the subsequent development of PD (p=0.03xxx). To better understand its relationship to PD, the QT interval was used to divide participants into 4 strata: <xxx msec (group 1 including 25% of subjects), xxx-xxx msec (50%), xxx-xxx (15%), and >=4xx msec (10% of subjects having the longest QT interval). Employing group I as the reference, the relative risk of PD increased with increasing QT duration as follows: Group 2 - RR=1.44 (95% CI= 0.93-2.25); Group 3 RR=1.24 (CI=0.68-2.28); group 4 RR=2.19 (CI=1.21-3.99, p=.01). Incidence rates (adjusted for age, caffeine intake, and smoking) of PD for the QT strata are shown in figure 1.

Electrocardiographic rcordings were also done on participants at the second (1967-70), third (1971-74), and 4th HHP (1991-93)examinations. Pearson correlation coefficients between the four measures of QT interval were: [exam 1]*[exam2], r=0.33; [exam1]*[exam3], r=0.26; [exam 2]*[exam 3], r=.29, [exam1]*[exam4], r=0.xx; [exam2]*[exam4], r=0.xx; [exam3]*[exam4], r=0.xx. Proportional hazards analyses were conducted using exam 2 and exam 3 QT measurements and methods similar to those mentioned above, and limiting the cases of PD to those diagnosed after more than 2 years following QT measurement. Although the associations were in the same direction as for the exam 1 QT interval (longer interval, higher incidence of PD), neither the exam 2 nor exam 3 QT interval was a statistically significant predictor of subsequent PD. Using logistic regression models and controlling for age, heart disease, and medication use we were unable to demonstrate a statistically significant association between prevalent PD cases (n=46) and concurrent QT interval at the fourth (1991-93) examination cycle, when subjects were 71-93 years of age. In addition we found no significant associations between heart rates at exam 1, 2, or 3 and the subsequent diagnosis of PD.

In order to examine a composite indicator that would take both the exam 2 and exam 3 measures into account, the following mutually exclusive strata were defined: 1) QT interval at or above the 75th percentile value at both the second and third HHP examinations (long-long, n=652), 2) QT interval shorter than the 25th percentile at both examination (short-short, n=625), 3) QT interval shorter than the 25th percentile at either but not both examinations (short, n= 1949), 4) QT interval at or longer than the 75th percentile at either but not both examinations (long, n=1750), 5) men with QT intervals between the 25th and 75th percentile values at both examinations, n=1741). Those few men whose qt intervals were below the 25th percentile at

one of the examinations and longer than the 75th percentile at the other examination (4.6% of participants) were assigned to the long or the short stratum, based on which value was most extreme. The results of these analyses, shown in figure x, indicate a significant difference in the relative risk for subsequent development of PD among men categorized as having long-long qt intervals (RR=2.4, 95% CI 1.035-5.575), compared with men classified as having short-short qt intervals. None of the other inter-stratum differences reached statistical significance.

One interpretation of these findings is that lengthening of the QT interval may occur as an early, preclinical sign of Parkinson's disease. Were this to be the case, one might reasonably expect the association to be stronger when the ECG was recorded closer to the time of diagnosis. The median interval between measurement of the QT interval at the first HHP examination, and the diagnosis of PD was 19.5 years. To assess the idea that qt lengthening might be a harbinger of the imminent development of PD, we assessed the predictive association of exam 1 QT length with incident PD in two subsets of the population, dividing the cases according to a lag interval less than or greater than the median lag time to diagnosis. As shown in table 1, part B, the predictive strength of a longer QT interval was most evident among persons in whom PD was diagnosed more than 19 years later.

An alternative interpretation is that the association with length of the QT interval might indicate a genetic or acquired predisposition to the development of PD, i.e., a constitutional risk factor. If this were true, cases in which the association was apparent might be different from other cases with regard to age of onset. Among the 137 cases identified in the HHP study cohort, the median age at the time of diagnosis of PD was 74.5 years. To assess this idea, we repeated our analyses after dividing the cases into two subsets, based on the age of diagnosis. As shown in part C of table I, the association of longer QT interval in midlife with the

subsequent development of PD was statistically significant only among cases diagnosed before age 75.

It is most important to consider the possibility that the observed relationship arose by chance, and that the apparent association of QT interval length with the pathogenesis of PD was spurious. To examine this possibility, we investigated the association of midlife QT interval length (1965-67) with a neuropathologic indicator of the degenerative process underlying PD: Lewy bodies in the pigmented brain stem nuclei at death. Comprehensive brain autopsy observations were available for 300 HHP/HAAS cohort members who died in the period 1991-2000, approximately 25-35 years after the QT interval was first measured. Lewy bodies were observed in substantia nigra and/or locus ceruleous sections in 52 of these decedents, including only 9 in whom PD had been diagnosed before death. The frequencies and extents of Lewy body pathology among the 300 decedents stratified according to the 1965-67 QT interval are illustrated in figure 2. Coefficients of correlation between the 1965-67 QT interval (corrected for heart rate) and pigmented brainstem Lewy bodies were 0.12 (Pearson; p=0.03) and 0.11 (Spearman, p=0.05x). After excluding the 9 individuals who had been diagnosed with PD the coefficients fell to 0.10 (Pearson, p=0.06) and 0.09 (Spearman, p=0.07). Additional analyses employed logistic regression methods were conducted with each autopsied decedent categorized as positive (n=52) or negative (n=248) for one or more Lewy bodies in the substantia nigra and/or locus ceruleus. Controlling for age at death and using the exam 1 QT interval as a continuous variable, its association with Lewy bodies was marginally significant (p=0.07). Additional logistic regression models were examined after stratifying decedents according to their 1965-67 QT interval (definitions as given for figure 1), controlling for age at the baseline exam and age at death. . With the shortest QT quartile defined as the reference category, odds

ratios for one or more Lewy body in the pigmented brainstem nuclei increased to 1.42 (95% CI 1.11-1.11) for men with intervals in the interquartile (25^{th}-75^{th} percentile) range , to 1.52 (CI 1.11-1.11) for intervals between the 75^{th} and 90^{th} percentile, and to 2.xx (CI 1.11-1.11) for men in the highest decile for QT length.

DISCUSSION

These findings indicate a statistically significant association of QT duration measured in midlife with the development of PD many years later, and a marginally significant association with Lewy bodies in the pigmented brainstem nuclei at death. Although the strongest associations were apparent among men with the longest intervals, they were not limited to men meeting diagnostic criteria for long QT syndrome, and were statistically significant only when comparing men having the longest interval with men having intervals shorter than the 25^{th} percentile. In our study population the association was statistically significant only mong men who developed PD at age 75 or younger, but was most apparent when the QT interval had been measured approximately two decades prior to diagnosis.

It is well accepted that some degree of autonomic dysfunction usually accompanies PD. {MARTIGNONI1986, TURKKA1987, TAKAHASHI1991, IEDA1999} Common abnormalities found in PD patients include orthostatic hypotension, postprandial hypotension, and constipation. Other findings include hypohidrosis, and uninary frequency. {NIIMI1999} In one study PD cases showed increased sweating with and without heat provocation {TURKKA1987} Resting skin temperature is slightly lower in PD cases compared to controls. {TAKAHASHI1991} Orthostatic hypotension and some of the other symptoms of autonomic

dysfunction have been observed to improve with administration of L-Dopa, {NIIMI1999,HAAPANIEMI2000} while others have pointed out that L-Dopa may cause orthostatic hypotension. {GOLDSTEIN2000, TAKAHASHI1991} Subnormal plasma concentrations of norepinepherine have also been found in PD {NIIMI1999} Although the autonomic nervous system contributes to regulation of the ECG QT interval, the precise relationship between autonomic mechanisms and QT interval has not been defined.

Changes in cardiac function have been studied in PD. Abnormalities include low ECG R-R interval variability (an indication of impaired parasympathetic activity) {TAKAHASHI1991} Heart rate at rest has been found to be lower, and the heart rate response to deep breathing and upward tilting is less robust in PD patients compared to controls. {HAAPANIEMI2000} Recently partial cardiac sympathetic denervation has been demostrated in PD. Using cardiac functional imaging, 20 of 29 PD cases had decreased septal 6-[18F]fluorodopamine-derived radioactivity compared to controls. Six of these subjects underwent right heart catheterization, and all had decreased extraction fractions of [3H] norepinephrine. Findings were unrelated to treatment with L-Dopa as well as duration, and severity of PD. {GOLDSTEIN2000} Another recent study showed that in early stages of PD there is a decrease in cardiac uptake of iodine-123-labelled metaiodobenzylguanidine (indicating sympathetic dysfunction) compared to controls and that this reduction was specific to PD (not present in multiple system atrophy, progressive supranuclear palsy or corticobasal degeneration). {TAKI2000} Iwanaga and colleagues discovered Lewy bodies in sympathetic neurons at autopsy within heart tissue in nine of eleven patients with PD and in all of seven participants identified with incidental Lewy bodies in brain. {IWANAGA1999}

The acute effect of sympathetic stimulation on QT interval has been studied using alpha and beta sympathetic receptor blockade. Findings have not been consistent. In an early study atropine (alpha blocker) decreased QT interval in healthy subjects who had received a beta-blocker but there was no effect on QT interval with the beta-blocker alone, {AHNVE1982} while another study demonstrated shortening of QT interval after beta-blocker administration. {SOLTI1989} A more recent study showed significant increases in QT interval with atropine. {ANNILA1993} The alpha-agonist isoproterenol has been shown to have no effect on QT interval in one study while atropine abolished normal standing associated QT shortening. {CUOMO1997} Long-term effects of autonomic stimulation on QT segment length are unknown.

There may be a link between the decreased nicotinic acetylcholine receptor (nAChR) activity seen in the central nervous system (CNS) in patients with PD and autonomic dysfunction in the heart. Autopsy studies have shown that in PD there is a marked reduction (more than 60%) in nicotinic (but not muscarinic) cholenergic receptors in various regions of brain including the cerebral cortex and the striatum. {JAMES1995, LAGNE1993, RINNE1991, PERRY1993} In addition there appears to be a direct relationship between degree of cognitive impairment in PD and reduction in nicotinic receptor binding. {AUBERT1992, WHITEHOUSE1998}

The nAChR is a ligand-gated cation channel. In addition to widespread distribution in the CNS, these receptors are located on peripheral postganglionic sympathetic nerve endings and in the adrenal medulla. Depolarization of the sympathetic nerve ending stimulates calcium influx through voltage-dependent calcium channels, and triggers exocytotic catecholamine release. Release of norepinepherine induces a beta-adrenoceptor mediated increase in heart rate and contractility, and an alpha-adrenoceptor-mediated increase in coronary vasomotor tone

accounting for changes in the cardiovascular system seen with acute exposure to nicotine. {HAASS1997} Chronic loss of nAChR activity within the autonomic nervous system could conceivably lead to imbalances causing long-term changes in cardiac function. Longer term effects of nicotine (outside the context of smoking) on the heart are unknown. It is noteworthy however that one longitudinal study has demonstrated a shortening of the QT interval in smokers. {KARJALAIEN1997}

Our findings, taken together with the information summarized above, suggest preclinical involvement of central and peripheral autonomic neurons in PD. The finding of a longer QT segment decades before the onset of clinical PD could be viewed either as indicators of preclinical disease, or as evidence of a constitutional risk factor. Since the time required for repolarization reflects ion pumping phenomena, it would seem reasonable to look

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Alternatively the QT segment length may be determined or modulated by excitable cell membrane mechanisms (such as ion channels) that might also influence the susceptibility of nigral neurons to excitotoxic, oxidative, or neurotoxic injury. These speculations and findings may have implications for early recognition of individuals at risk for PD, and/or may point to fundamental metabolic factors that predispose dopaminergic neurons in the substantia nigra to injury and death.

Table 1

	BASELINE QT INTERVAL			
	<392 msec	392-424 msec	425-438 msec	>=439 msec
ENTIRE COHORT				
N (PD/total)	28 / 2053	74 / 3966	17 / 1206	18 / 765
Odds Ratio	1.0 (ref)	1.43	1.13	2.09
95% c.i.		0.92-2.11	0.61-2.08	1.15-3.78
TIME TO PD DIAGNOSIS				
<=19 yrs				
N	13 / 2053	38 / 3966	8 / 1206	8 / 765
OR	1.0 (ref)	1.49	1.13	1.83
95% c.i.		0.79-2.80	0.47-2.73	0.76-4.44
> 19 yrs				
N	15 / 1614	36 / 2996	9 / 864	10 / 511
OR	10. (ref)	1.36	1.11	2.33
95% c.i.		0.74-2.48	0.47-2.62	1.05-5.19
AGE AT PD DIAGNOSIS				
<75 years				
N	15 / 2053	39 / 3966	7 / 1206	13 / 765
OR	10. (ref)	1.41	0.95	2.93
95% c.i.		0.77-2.55	0.39-2.34	1.39-6.16
>= 75 years				
N	13 / 1597	35 /2990	10 / 848	5 / 504
OR	1.0 (ref)	1.48	1.32	1.20
95% c.i.		0.78-2.79	0.57-3.10	0.43-3.38

QT interval groups were defined so that the first stratum included 25% of the cohort, the next interval included the interquartile range, the third included those with intervals between the 75th and 90th percentile, and the final stratum included the 10% with longest intervals.

All results are based on Cox proportional hazards models controlling for age, caffeine intake in a 24 hour period prior to examination, usual cigarettes per day, and accrued pack years of cigarettes smoked at baseline.

Table 1. Association of length of the QT interval measured at baseline (1965-67) with Parkinson's disease developing during 3 decades of follow-up.

	Exam 2/ Exam 3 composite QT Interval strata				
	Short-short	short	intermediate	long	long-long
N (PD/total)	8 / 625	35 / 1948	34 / 1737	22 / 1748	16 / 651
Odds Ratio	1.0 (ref)	1.45	1.63	1.10	2.20
95% c.i.		0.67-3.12	0.75-3.52	0.49-2.48	0.94-5.14

Interval strata based on QT quartiles determined at the 2nd and 3rd exams: short-short = shortest quartile at both exams; long-long = longest quartile, both exams; short = short quartile, one exam only; long = long quartile, one exam only. Endpoint: PD cases developing more than 2 years following the 3rd exam.

Analysis by Cox proportional hazards, controlling for age, caffeine intake, and accrued pack years of cigarettes smoked at exam 1.

Table 2. Association of length of the QT interval measured at the second (1967-69) and third (1971-74) examinations with Parkinson's disease developing during the subsequent 2 decades of follow-up.

	BASELINE QT INTERVAL			
	<392 msec	392-424 msec	425-438 msec	>=439 msec
all autopsies available				
N (Lewy+ / autopsies)	9 / 83	27 / 143	10 / 54	6 / 23
Odds Ratio	1.0 (ref)	1.82	1.79	3.04
95% c.i.		0.81-4.12	0.67-4.79	0.95-9.77
autopsies excluding PD cases				
N (Lewy+ / autopsies)	8 / 81	22 / 136	9 / 53	4 / 21
Odds Ratio	1.0 (ref)	1.66	1.74	2.25
95% c.i.		0.79-3.95	0.62-4.88	0.60-8.44

QT intervals determined 1965-67. Autopsies done 1991-2000. Lewy bodies identified in the pigmented brainstem nuclei using H and E and anti alpha synuclein stains. Odds ratios from logistic regression models controlling for age at death and age at baseline.

Table 3. Association of QT interval length measured at baseline with Lewy bodies observed in the substantia nigra and/or locus ceruleus at autopsy 26-35 years later.

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Midlife adiposity and the future risk of Parkinson's disease

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Abstract—*Background:* Evidence suggests that nigrostriatal system disorders are associated with PD and adiposity. Whether patterns of adiposity coexist or predate clinical PD is unknown. This report examines the relation between midlife adiposity and the risk of PD. *Methods:* Measurement of adiposity occurred from 1965 to 1968 in 7,990 men in the Honolulu Heart Program (aged 45 to 68 years and without PD). Adiposity measures included body mass index (BMI), subscapular skinfold thickness (SSF), and triceps skinfold thickness (TSF). Follow-up for incident PD occurred over a 30-year period. *Results:* During the course of follow-up, PD was observed in 137 men. Among the measures of adiposity, age-adjusted incidence of PD increased threefold from 3.7/10,000 person-years in the bottom quartile of TSF (1 to 5 mm) to 11.1/10,000 person-years in the top quartile (11 to 32 mm, $p < 0.001$). Effects of TSF on PD were independent of cigarette smoking, coffee consumption, physical activity, daily caloric and fat intake, and the other measures of adiposity ($p < 0.001$). Whereas rates of PD were lowest in the bottom quartile of BMI and SSF vs higher quartiles, associations with PD were weaker than they were for TSF. The effect of TSF on clinical onset before age 65 years was similar to the effect that was observed in later life. *Conclusions:* Increased triceps skinfold thickness measured in midlife is associated with an elevated risk of future PD. Whether patterns of adiposity reflect a unique metabolic pathology in individuals at a high risk of PD warrants further study.

NEUROLOGY 2002;59:1051–1057

The cardinal signs of PD are due in large part to the loss of dopamine-producing neurons in the pars compacta region of the substantia nigra.[1] Nerve cell loss in other regions of the brain, including influences on the autonomic nervous system, is also known to occur.[1-7] Evidence for an effect of complex nervous system interactions involving autonomic dysfunction on appetite regulation and energy metabolism,[8] as well as recent observations that obesity in humans is related to the depletion of striatal dopamine receptor

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Supported by a grant from the United States Department of the Army (DAMD17-98-1-8621), by the NIH (National Institute on Aging Contract N01-AG-4-2149 and grant 1-R01-AG17155-01A1, National Heart, Lung, and Blood Institute Contract N01-HC-05102, and a National Institute of Neurological Disorders and Stroke grant 1-R01-NS41265-01), and by Department of Veterans Affairs medical research funds.

The information contained in this article does not necessarily reflect the position or the policy of the Government, and no official endorsement should be inferred.

Received April 15, 2002. Accepted in final form June 27, 2002.

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availability,[9] suggests that nigrostriatal system disorders are associated with PD and adiposity. Whether these pathologic processes coexist or whether characteristic patterns of adiposity can predate clinical PD and its early motor symptoms is unknown. Such processes could have associations with specific forms of obesity and contribute to the complexity and heterogeneity in body fat among individuals and to its wide variation in response to exercise, diet, and other interventions. The purpose of this report is to examine the association between measures of adiposity observed in middle-adulthood and the future risk of PD based on 30 years of follow-up of a cohort of asymptomatic men enrolled in the Honolulu Heart Program.

Materials and methods. *Study sample.* From 1965 to 1968, the Honolulu Heart Program began following 8,006 men of Japanese ancestry living on the island of Oahu, Hawaii, for the development of cardiovascular disease.[10-12] At the time of study enrollment, subjects were aged 45 to 68 years. Initial screening consisted of a baseline physical examination and documentation of cardiac and neurologic conditions to identify prevalent cases of cardiovascular disease. Procedures were in accordance with institutional guidelines and approved by an institutional review committee. Informed consent was obtained from the study participants.

Since the beginning of the Honolulu Heart Program, surviving members participated in repeat examinations and were tracked for morbidity and mortality outcomes through a comprehensive system of surveillance that included a review of all hospital discharges, death certificates, and autopsy records. For this report, follow-up for incident PD began at the time of study inception (1965 through 1968). After excluding 14 men with missing measures of adiposity and two men with prevalent PD, 7,990 remained available for follow-up.

Measurement of adiposity and confounding information. At the time when follow-up began, body mass index (BMI) was used as a standard measure of overall adiposity (weight in kg/height in m^2). Measures of central and peripheral adiposity included subscapular skinfold thickness (SSF) and triceps skinfold thickness (TSF). For both SSF and TSF, skinfold thicknesses were recorded to the nearest millimeter on the left side in the standing position using Lange calipers (Cambridge Instruments). Measurement of SSF was taken 4 cm below the angle of the scapula. For TSF, arms hung vertically with muscles relaxed while measurements were taken over the triceps muscle midway between the axilla and the elbow.

Other confounding information observed at the time of study enrollment and known to be related to PD included age, pack-years of cigarette smoking, and intake of coffee.[13,14] Data on the intake of caloric and dietary fat were also collected. Information on coffee consumption and daily caloric and fat intake was obtained by a dietitian based on 24-hour dietary recall methods and validated against 7-day diet records in a subset of the original cohort.[15] Assessment of physical activity was also made through the measurement of a physical activity index to quantify overall metabolic output during a typical 24-hour period.[16-19] Low levels of the physical activity index have been shown to be associated with an increased risk of coronary heart disease and stroke.[16-19]

PD case finding and diagnosis. For this report, 30 years of follow-up data were available on incident PD after collection of information on adiposity (1965 through 1968). Before 1991, cases of PD were identified through a review of all hospital records of study participants for new and preexisting diagnoses of PD, an ongoing review of all Hawaii death certificates, and a review of medical records at the offices of local neurologists for all cohort members suspected to have PD.

After 1991, study participants were screened for PD at examinations that occurred from 1991 to 1993. During this time, all subjects were questioned about a diagnosis of PD and the use of PD medications by a structured interview. Study participants received further screening by a technician trained to recognize the clinical signs of parkinsonism (including gait disturbance, tremor, and bradykinesia). Those with a history or sign of parkinsonism were referred to a study neurologist who administered standardized questions about symptoms and the onset of parkinsonism, previous diagnoses, and medication use, followed by a comprehensive and standardized neurologic examination. A diagnosis of PD was made by the study neurologists according to published criteria without access to the risk factor data examined in this report.[20] These required that the subject have the following: 1) parkinsonism (e.g., bradykinesia or resting tremor combined with rigidity or postural reflex impairment); 2) a progressive disorder; 3) any two of a marked response to levodopa, asymmetry of signs, asymmetry at onset, or initial onset tremor; and 4) absence of any etiology known to cause similar features. Cases of parkinsonism related to progressive supranuclear palsy, multisystem atrophy, cerebrovascular disease, drug-induced parkinsonism, postencephalitic parkinsonism, or post-traumatic parkinsonism were not included among the cases of PD. During repeat examinations that were given from 1994 to 1996 and from 1996 to 1998, subjects were again asked about a diagnosis of PD and the use of PD medications. Medical records were further reviewed by the study neurologists who applied the same published criteria used earlier in making a diagnosis of PD.[20] Further description of the diagnosis of PD is described elsewhere.[13,21]

Statistical methods. Crude and age-adjusted incidence rates of PD in person-years were estimated according to ranges of BMI, SSF, and TSF based on the 30 years of follow-up in the 7,990 men who were examined from 1965 through 1968.[22] Age-adjusted risk factors across approximate quartiles of each adiposity measure were also derived.[22] To test for an independent effect of BMI, SSF, and TSF on the risk of PD, proportional hazards regression models were examined.[23] Adjustments were made for age, pack-years of cigarette smoking, coffee consumption, daily caloric and fat intake, and the other measures of adiposity. While BMI, SSF, and TSF were modeled as continuous risk factors, relative risks of PD (and 95% CI) were also estimated comparing the risk of PD between men in each of the top three quartiles of an adiposity measure to those in the bottom quartile. All reported p values were based on two-sided tests of significance.

Results. The average age at study enrollment (1965 through 1968) of the 7,990 men was 54 years (range, 45 to 68). During the 30 years of follow-up, 137 developed PD.

Table 1 *Incidence of PD by quartile of BMI, SSF, and TSF measured at the time of study inception (1965–1968)*

Quartile (range)	Sample size	Incident PD cases	Incidence of PD, rate/10,000 person-years	
			Unadjusted	Age-adjusted
BMI, kg/m^2				
1st (14.3–21.7)	1,996	20	4.3	4.1
2nd (21.8–23.8)	1,991	41	8.5†	8.3†
3rd (23.9–25.8)	2,011	45	9.1‡	9.2‡
4th (25.9–39.9)	1,992	31	6.5	6.8
Test for trend, p value*			0.243	0.116
SSF, mm				
1st (2–10)	1,660	14	3.6	3.4
2nd (11–16)	2,656	58	9.0‡	8.9‡
3rd (17–21)	1,789	30	6.8	6.9†
4th (22–51)	1,885	35	7.7†	8.2‡
Test for trend, p value*			0.195	0.098
TSF, mm				
1st (1–5)	1,901	17	3.8	3.7
2nd (6–7)	2,205	35	6.5	6.6
3rd (8–10)	2,298	44	7.9‡	7.8‡
4th (11–32)	1,586	41	10.8§	11.1§
Test for trend, p value*			<0.001	<0.001

* Based on modeling the adiposity measure as a continuous variable.
† Excess risk of PD vs men in the 1st quartile ($p < 0.05$).
‡ Excess risk of PD vs men in the 1st quartile ($p < 0.01$).
§ Excess risk of PD vs men in the 1st quartile ($p < 0.001$).

BMI = body mass index; SSF = subscapular skinfold thickness; TSF = triceps skinfold thickness.

The average age at the time of diagnosis was 73 years (range, 54 to 89) and the average time to a diagnosis was 19 years (range, 2 to 30).

Incidence of PD by quartile of BMI, SSF, and TSF is shown in table 1. For each adiposity measure, age-adjusted incidence of PD was lowest in the first quartile as compared to quartiles that were higher. Differences in the risk of PD across the top three quartiles of BMI and SSF were not apparent. In contrast, the age-adjusted incidence of PD rose consistently from 3.7/10,000 person-years in men in the bottom quartile of TSF (1 to 5 mm) to a threefold excess (11.1/10,000 person-years) in those in the top quartile (11 to 32 mm, $p < 0.001$).

Associations between potential factors that could confound the relation between an adiposity measure and the risk of PD are described in table 2. Mean ages and age-adjusted covariates that were measured at the time of study enrollment are provided across the quartiles of BMI. Comparisons across quartiles of SSF and TSF were similar.

Among the factors, age, pack-years of cigarette smoking, the percent of men who were current smokers, and the physical activity index declined with increasing BMI ($p < 0.05$). The percent of men who were past smokers increased with BMI ($p < 0.001$). There was no clear relation between the daily intake of coffee, calories, and fat across the ranges of BMI. As expected, there was a positive association between BMI and the other adiposity measures ($p < 0.001$).

After adjusting for the possible confounding effect of these other factors, only the association between TSF and PD remained significant. Table 3 provides the results of this latter finding. After adjustment for age, pack-years of smoking, coffee consumption, physical activity, and daily caloric and fat intake (column A), the relative risk of PD increased from 1.5 to 2.5 for men in the second to top quartile of TSF as compared to those in the first quartile. As a continuous risk factor, the rise in PD incidence with increasing TSF was significant ($p < 0.001$).

To help determine if the effect of TSF on the risk of PD could be independently attributed to the peripheral location of body fat, additional adjustments were made for BMI and SSF (markers of overall and central adiposity). As seen in column B of table 3, findings suggest that the effect of TSF on PD is independent of the other adiposity measures. The increase in the observed relative risks (column B) as compared with when the effects of BMI and SSF were ignored (column A) is largely due to a small excess of PD in men with an elevated TSF who also had low levels of BMI and SSF. Tests for interaction effects between the adiposity measures on the risk of PD, however, were not significant.

The figure (top panel) further describes the association between TSF and the age-adjusted incidence of early and late PD onset (<65 and ≥65 years of age). Overall, early onset of PD occurred in 19 men (2.3/10,000 person-years), whereas late onset occurred in 118 men (10.7/10,000 person-years). Although the number of early onset cases is small, risk comparisons among the quartiles of TSF were not appreciably different from those made for the later-onset cases. The association between TSF and PD was also not significantly related to the time elapsed from the measurement of TSF to the diagnosis of PD (see the figure, bottom panel). In the first 15 years of follow-up, 42 men were diagnosed with PD (3.7/10,000 person-years), whereas 95 cases were diagnosed in the second 15 years of follow-up (11.9/10,000 person-years). As with early- and late-onset PD, effects of TSF on the risk of PD were similar for diagnoses that occurred during each 15 years of follow-up. Similar findings were also observed within other periods of follow-up.

Discussion. Although loss in body fat is common in patients with clinical PD,[24,25] reported findings have been limited to cross-sectional and case-control studies with uncertain recall and timing of anthropometric histories. Rarely are different adiposity measures (BMI, SSF, and TSF) available for the assessment of their effects on future disease. We are not aware of another study that has been able to prospectively examine the association between midlife adiposity and the future risk of PD. A major strength of the current report also includes the measurement of adiposity following a standardized protocol well before the development of PD. Because

Table 2 Average age and age-adjusted covariates according to quartile of BMI measured at the time of study inception (1965–1968)

Covariates	Quartile of BMI			
	1st	2nd	3rd	4th
Age, y†	55.4 ± 5.8	54.6 ± 5.5	54.0 ± 5.4	53.7 ± 5.5
Coffee intake/d, dL	4.0 ± 3.6	4.0 ± 3.8	4.0 ± 3.7	3.9 ± 3.8
Pack-years of smoking*	33.9 ± 28.4	31.7 ± 29.7	30.0 ± 29.6	31.0 ± 31.2
Current smoking status, %				
Past‡	19.3	25.7	29.3	27.0
Current†	59.4	46.6	40.6	40.4
Physical activity index†	33.4 ± 4.5	32.9 ± 4.5	32.5 ± 4.4	32.4 ± 4.6
Kilocalorie intake/d	2212 ± 653	2245 ± 660	2231 ± 680	2178 ± 715
Fat intake/d, g	75.2 ± 32.7	77.7 ± 32.9	78.0 ± 34.0	77.0 ± 34.8
SSF, mm‡	10.0 ± 3.6	15.0 ± 4.6	18.2 ± 5.1	22.7 ± 6.2
TSF, mm‡	5.6 ± 2.2	7.6 ± 2.7	8.6 ± 2.9	10.1 ± 3.8

Values are mean ± SD unless otherwise indicated.

* Covariate declines with increasing BMI ($p < 0.01$).
† Covariate declines with increasing BMI ($p < 0.001$).
‡ Covariate increases with increasing BMI ($p < 0.001$).

BMI = body mass index; SSF = subscapular skinfold thickness; TSF = triceps skinfold thickness.

subjects did not have PD when follow-up began, effects of medication for PD on patterns of adiposity are also absent.

These findings suggest that adiposity in middle-adulthood is related to an increased risk of PD in later life. Although PD risk was consistently less in men in the bottom vs higher quartiles of each adiposity measure, associations were strongest for TSF. Here, risk of PD increased consistently with increasing TSF levels after accounting for other risk factors effects, including the simultaneous control for BMI and SSF. In addition, associations were similar for early and late onset of PD and for diagnoses made 15 years beyond the time of adiposity measurement.

Table 3 Estimated risk factor–adjusted relative risk of PD in men in the top three quartiles of TSF as compared to men in the 1st quartile

Quartile comparison	Risk factor–adjusted relative risk	
	A*	B†
2nd vs 1st	1.5 (0.9–2.8)	1.6 (0.9–3.0)
3rd vs 1st	1.8§ (1.0–3.2)	2.0§ (1.1–3.6)
4th vs 1st	2.5¶ (1.4–4.4)	2.8¶ (1.4–5.6)
Test for trend, p value‡	<0.001	<0.001

Values are relative risk (95% CI).

* Adjusted for age, pack-years of smoking, coffee intake, physical activity index, and daily caloric and fat intake.
† Adjusted for age, pack-years of smoking, coffee intake, physical activity index, daily caloric and fat intake, and the other measures of adiposity.
‡ Based on modeling TSF as a continuous variable.
§ Excess risk of PD vs men in the 1st quartile ($p < 0.05$).
¶ Excess risk of PD vs men in the 1st quartile ($p < 0.01$).

TSF = triceps skinfold thickness.

The relation between TSF and PD also seemed to persist for repeated measurements that were made from 1991 through 1993, although statistical testing may be limited because of reductions in available follow-up. During the later examination, measurements of BMI, SSF, and TSF were available in 3,512 surviving members of the original cohort aged 71 to 93 years without PD. Among this group, TSF tended to increase from baseline (1965 through 1968) values by an average of 2.2 ± 4.0 mm, whereas SSF and BMI declined modestly (−0.3 ± 6.7 mm and −0.5 ± 2.6 kg/m^2). Although changes in body composition are expected to occur with age, each baseline measure was positively and strongly predictive of the later (1991 through 1993) measure ($p < 0.001$).

In the remaining years of follow-up, 27 men developed PD (20.3/10,000 person-years). Age-adjusted incidence of PD for men in the top quartile of TSF (12.5 to 34 mm) was 34.3/10,000 person-years vs 16.4/10,000 person-years in those who were leaner (2 to 12 mm). The incidence of PD continued to rise significantly with increasing TSF after adjustment for age, BMI, and SSF ($p = 0.013$). Relations between the other adiposity measures and PD were positive but not significant. In addition, after controlling for TSF at the time of study enrollment (1965 through 1968), risk of PD rose with increasing TSF as the cohort aged, also independent of the other adiposity measures ($p = 0.048$).

Explanations for the observed findings in the Honolulu Heart Program are unclear, particularly for the long-term preclinical effects of adiposity on cases

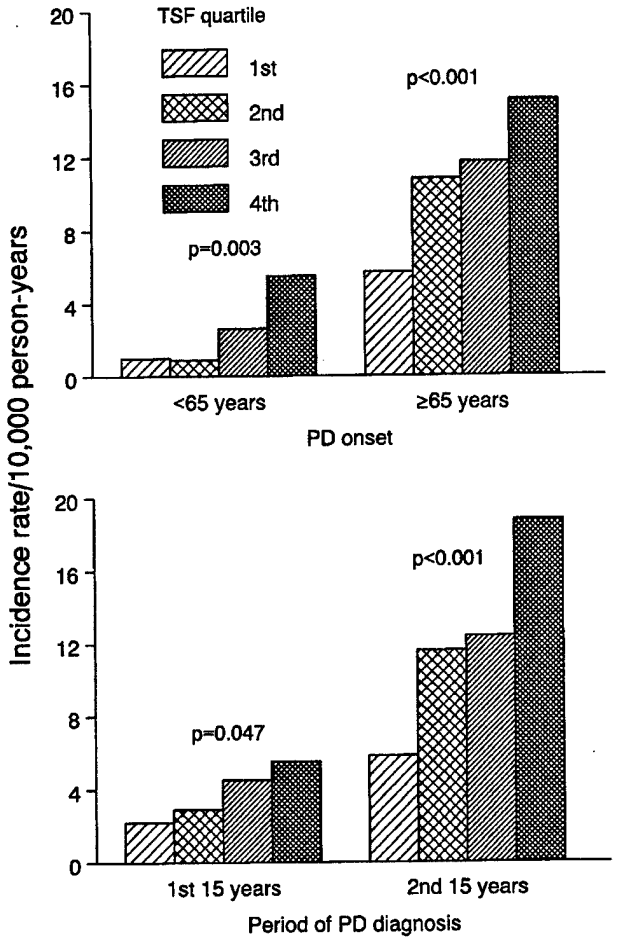

Figure. Age-adjusted incidence of early and late PD onset (<65 and ≥65 years of age) and the incidence of PD in the first and second 15 years of follow-up according to quartile of triceps skinfold thickness (TSF) measured at the time of study inception (1965 through 1968). p *Values represent a test for trend based on modeling TSF as a continuous variable.*

of PD that were diagnosed well after the time of study enrollment. Excesses in adiposity could have been the consequence of physical inactivity induced by bradykinesia and undiagnosed early PD, reflecting an insidious pathogenic process with a long latency period of more than 15 years in many instances. Such a long latency period is in contrast to the estimated 3- to 6-year preclinical period based on neuroimaging and neuropathology studies in PD.[26,27] Although further explanation is needed, the long interval between recorded measures of adiposity and the diagnosis of PD in our study may provide some insights into the pathogenesis of PD and related histologic changes that begin as early as 25 years of age.[28] Whether the mechanisms associated with the long-term preclinical effects observed in the current report are different from those that result in weight loss in patients with clinical PD is unknown.[24,25] In the current report, physical activity also had no effect on the risk of PD, nor did it modify the observed relation between PD and the measures of adiposity.

Adipose tissue is also known as an important site of estrogen metabolism.[29,30] In light of the suggestion that endogenous estrogen in women may have a neuroprotective effect against PD,[31] it might be expected that the risk of PD could be reduced in those who are obese. The neuroprotective effect of estrogen, however, has not been clearly established. Others have found no such effect and further hypothesize that estrogen may be antidopaminergic.[32] Even in the presence of a protective effect of estrogen, estrogen levels resulting from excesses in adiposity in men may fail to reach critical levels to allow for the appearance of an inverse relation between body fat and PD. Long-term exposure to estrogen concentrations that are normally seen in premenopausal women may also be required. Estrogen metabolism may further vary according to the location of adipose tissue,[30] particularly between peripheral (TSF) and central (SSF) body fat. Whether findings from the Honolulu Heart Program can also apply to women warrants further study.

In addition, extensions to other population segments is also unknown, although the rate of PD described in the current report is in general agreement with rates that have been observed in Europe and the United States.[21,33] Nevertheless, men in the Honolulu Heart Program are unique. For example, early childhood experiences were often difficult. Study participants were either immigrants or the progeny of immigrants from Japan who migrated to Hawaii as contract laborers to serve in the sugar and pineapple industries. As a possible consequence of these experiences at an important time of physical development, subjects tended to be smaller than men of similar age in the United States.[34]

There also exists the possibility that mortality from other causes could have resulted in a poor estimate of the true effect of adiposity on PD, although such an effect is likely to be small. The best description of the true association between TSF and PD might actually appear in the left side of the top panel of the figure (for PD cases diagnosed <65 years). In this instance, competing risks occur too infrequently in this long-lived sample to account for the pattern of association that was observed between TSF and PD. Even within the first 15 years of follow-up, the effect of early mortality from other causes is likely to be modest. Early mortality in the top or bottom quartiles of TSF also does not explain the findings observed in this report. After adjustment for age and the other risk factors, including BMI and SSF, excluding men in the top and bottom quartiles of TSF failed to alter the observed association between TSF and PD ($p = 0.0291$ and $p = 0.007$ when men in the top and bottom quartiles were excluded). Duplicate analyses for BMI and SSF also failed to alter the observed associations that these body composition measures have with PD.

Although corroborating data are limited, a recent

animal study suggests that the association between adiposity and PD could be due to an increased susceptibility to environmental factors that lead to PD. In transgenic mice with genetically determined obesity, increased vulnerability to the neurotoxicants methamphetamine and kainic acid was associated with a greater decrease in levels of striatal dopamine and tyrosine hydroxylase and to elevated levels of glial fibrillary acidic protein, a sensitive indicator of neuronal damage.[35] Body fat may also act as a reservoir for lipid-soluble neurotoxins that selectively damage dopamine-producing neurons in the substantia nigra. Regional differences in fatty tissue turnover and neurotoxin release from these regions may also explain the stronger association of peripheral body adiposity (TSF) to PD as compared to overall (BMI) and central (SSF) adiposity.

Obesity could also be directly linked with derangements in dopaminergic systems that increase the risk of PD. Recently, obesity in humans has been associated with a decrease in dopamine receptor availability in a study using [c-11] raclopride to measure D_2 dopamine receptors with PET.[9] Increases in appetite and weight have also been associated with drugs that block dopamine D_2 receptors,[36,37] whereas treatment with levodopa is often associated with weight loss and appetite suppression.[38] It is possible that decreased D_2 receptors in obese individuals could lead to compensatory increases in dopamine turnover, consequent increases in oxidative metabolites, and eventually, to an increase in oxidative stress and neuronal death.

High caloric and fat intake, including the intake of dietary cholesterol, has also been observed in patients with PD,[39,40] although an association between these dietary items (including iron and animal fat) and the future risk of PD was not observed in the Honolulu sample. There also exists the possibility that such effects were not apparent because of the underreporting of dietary intake in subjects who were overweight.[41,42] Such underreporting in the current study, however, does not seem to explain the relation that was observed between adiposity and PD because excesses in PD were also observed in the leaner men in the second quartile of each body composition measure as compared to the first quartile (see table 1).

Although clinical implications are difficult to address based on findings from the current report, identifying patterns of adiposity that predate clinical PD could suggest that subtle PD processes have the potential for being recognized before the emergence of motor symptomatology. Combining information on adiposity with other factors, such as a positive family history or early signs of developing movement abnormalities, could have some uses for identifying high-risk individuals for future PD. In light of the evidence that pathologic processes in PD may include effects on adiposity,[8,9,36-40] further studies of susceptibility and environmental factors that may increase the risk of PD in individuals with characteristic patterns of adiposity appear to be warranted.

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APPENDIX H

Please select Print from the file menu to print your Abstract.

The *Movement* Disorder Society

Filename: 950998
Presenting Author: George W Ross
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Address: 846 S. Hotel St. , Suite 307
City/State/Zip/Country: Honolulu, Hawaii, 96813, United States
Phone: 808 524-3595 **Fax:** 808 524-4315 **E-mail:** ross@phri.hawaii-health.com
Category: Parkinson's Disease
Presentation Video: My abstract will not require accommodations for a video presentation
Junior Awards: Please do not consider my abstract for an MDS Junior Award
Keywords: Parkinson's disease, reaction time, Lewy body
Disclosure:
University Monies: None
Grant Monies: Grant Monies are indicated. Details: National Institute of Neurological Disorders and Stroke[br]Department of Veterans Affairs Medical Research Funds[br] National Institute on Aging[br]Department of Defense
Industry: None
Government: None
Self: None
Possible author conflict of interest: None

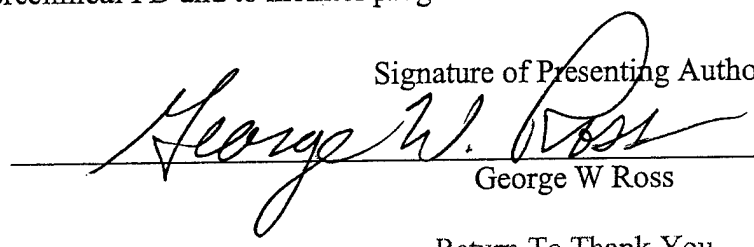
Title: Performance on a computerized reaction time test predicts incidental Lewy bodies

Authors: George W Ross, Honolulu, HI*, Helen Petrovitch, Honolulu, HI, Robert D Abbott, Charlottesville, VA, Caroline M Tanner, Sunnyvale, CA, Daron G Davis, Lexington, KY and James S Nelson, Honolulu, HI. , et al.

Body/Text:
Objective: to examine the association of performance on the 3RT Test, a computer administered test of simple and choice reaction times, with the presence of incidental Lewy bodies (LB) in the substantia nigra (SN) or locus ceruleus (LC) of decedent participants from the Honolulu-Asia Aging Study (HAAS) who did not have clinical Parkinson's disease (PD).
Background: In early, untreated Parkinson's disease, simple reaction time is impaired while choice reaction time is affected later. Accrued evidence supports the idea that the pathogenic process leading to PD is commonly active well before the clinical illness is diagnosed. Simple reaction time might be useful for the identification of persons in the preclinical phase when the neuropathologic lesions are established but have not progressed sufficiently to cause recognizable signs and symptoms. We hypothesized that persons with slow simple reaction time would be more likely to have incidental LB in the SN or LC at autopsy. Methods: The 3RT Test was administered to all subjects at the 1994-96

examination of the HAAS, a prospective study of neurodegenerative diseases of aging in a cohort of Japanese-American men living in Hawaii and born 1900-1919. The brains of 96 men without PD who underwent RT testing were examined for LB in the LC or SN.
Results: Among the 96 brains examined, 8 had incidental LB. The mean interval from RT testing to death was 2.2 years (median 2.3 years, range = 0.01-4.6). The percent of brains with incidental LB increased consistently from 0% (0/24, fastest quartile) to 16.7% (4/24, slowest quartile) among subjects classified into four subsets according to simple reaction time ($p=0.037$ for age adjusted test for trend). This relationship was not significant for choice reaction time.
Conclusions: Slow reaction time predicted incidental LB. The 3RT might help to identify preclinical PD and to monitor progression to clinical illness in selected subjects.

Signature of Presenting Author:



George W Ross

Return To Thank You

Afternoon Session

Session No. 64
MOVEMENT DISORDERS: PARKINSONISM

Thursday, May 4, 2000

2:00 PM–3:30 PM

Room No. 6A

Co-chairs: Bob Hauser, Tampa, FL
Niall Quinn, London, United Kingdom

S64.001

2:00 PM

Association of Olfactory Dysfunction with Presence of Lewy Bodies in the Substantia Nigra or Locus Ceruleus at Autopsy: A Prospective Cohort Study

George W. Ross, Honolulu, HI, Caroline M. Tanner, Sunnyvale, CA, Robert D. Abbott, Charlottesville, VA, Helen Petrovitch, Honolulu, HI, Daron G. Davis, Lexington, KY, James Nelson, New Orleans, LA, William Markesbery, Lexington, KY, John Hardman, Lon R. White, Honolulu, HI, Dan Foley, Bethesda, MD

OBJECTIVE: To determine the association of olfactory dysfunction during late life with incidental Lewy bodies in the substantia nigra (SN) or locus ceruleus (LC) at autopsy.

BACKGROUND: Olfactory dysfunction has been associated with Parkinson's disease (PD), however, its diagnostic significance is unknown. If olfactory dysfunction is associated with incidental Lewy bodies, then an olfactory deficit may serve as a sign of those at risk or an early sign of disease.

DESIGN/METHODS: The brains of 108 elderly Japanese-American men without PD who were participants in the population based, longitudinal Honolulu Heart Program/Honolulu-Asia Aging Study were examined for the presence of Lewy bodies in two brainstem pigmented nuclei, the substantia nigra and the locus ceruleus, with hematoxylin—eosin stains. Olfaction was measured prospectively (1991–94 and 1994–96 cohort examinations) using the 12 item University of Pennsylvania Smell Identification Test (UPSIT). Logistic regression methods were used to examine the association of olfactory dysfunction and incidental Lewy bodies.

RESULTS: Among the 108 brains examined, 16 (14.8%) had Lewy bodies in either the SN or LC. None of the 16 individuals with Lewy bodies had clinical PD. Higher UPSIT scores (better olfactory function) at the examination nearest to death were inversely associated with incidental Lewy bodies after adjustment for age (Odds Ratio = 0.745; P=0.0085). This was not seen for the earlier examination (on average 5 years prior to death) (Odds Ratio = 0.921; P=0.331).

CONCLUSIONS: Olfactory dysfunction within four years of death was predictive of incidental Lewy bodies in the brainstem pigmented nuclei, but this relationship was not seen for longer intervals. Incidental Lewy bodies are proposed to be the pathologic correlate of presymptomatic PD. The association between impaired olfaction and incidental Lewy bodies suggests that impaired olfaction may precede the motor signs of PD. If this is correct, olfactory testing may prove to be a useful screening tool to detect those at risk for developing PD who might benefit from neuroprotective therapies.

Supported by: Department of Defense Grant DAMD17–98-1–8621, the Department of Veterans Affairs Merit Review Grant 9003–0004, National Institutes of Health, National Institute on Aging contract N01-AG-4–2149, and National Heart, Lung, and Blood Institute contract N01-HC-05102

S6[illegible]

2:15 PM

Disease Progression of Dementia with Lewy Bodies: A Clinicopathological Study

K. Seppi, G. K. Wenning, Innsbruck, Austria, K. Jellinger, Vienna, Austria, E. Luginger, G. Ransmayr, Innsbruck, Austria, K. R. Chauduri, London, United Kingdom, A. McKee, Boston, MA, M. Verny, Paris, France, S. E. Daniel, London, United Kingdom, W. Poewe, Innsbruck, Austria, I. Litvan, Bethesda, MD

OBJECTIVE: To determine disease progression of patients with postmortem confirmed dementia with Lewy bodies (DLB).

BACKGROUND: There are few small scale published reports evaluating disease progression in postmortem confirmed DLB.

DESIGN/METHODS: Sixty-two DLB cases satisfying the neuropathological consensus criteria proposed by McKeith et al. (1996) were selected from the research and clinical files of tertiary medical centers in Austria, France, United Kingdom, and the United States. Disease progression in these well-documented cases was studied at three time points during the disease course: disease onset, first and last neurological evaluation.

RESULTS: There were 26 male and 36 female patients, average age at symptom onset was 69.4±10.0 years and average disease duration was 7.5±6.6 years. Progressive memory loss was the most common initial symptom occurring in 45% of the patients followed by parkinsonian features in 39%. First and last neurological visits took place on average 38 and 87 months after symptom onset. Frequent clinical features present at the first neurological visit included memory impairment (69%), bradykinesia (59%), gait disturbance (57%), and frontal lobe behavior (55%). Paranoid delusions (43%), fluctuating cognition (37%), and visual hallucinations (33%) were less common. At last neurological visit, the frequency of presenting motor and neuropsychiatric features generally increased. However, a substantial number of patients never exhibited classical features associated with DLB such as fluctuating cognition and hallucinations. Frequent additional features that had emerged at last evaluation included dysarthria (78%), urinary incontinence/retention (71%), and dysphagia (65%). Orthostatic, cerebellar, pyramidal, and oculomotor features were uncommon, and if present, they were only mild.

CONCLUSIONS: Our data demonstrate that the characteristic clinical features of DLB may evolve over a time course of up to 7.5 years. The association at early stages of severe memory deficit, parkinsonism, or gait disturbance should increase our index of suspicion of DLB. Even at late disease stages cardinal features of DLB such as fluctuating cognition, paranoid delusions, and visual hallucinations were absent in many patients. In view of our findings, currently used clinical diagnostic criteria for DLB should be modified.

Supported by: BMWV Austria (Project-number: GZ 70038/2-Pr/4/98)

S64.003

2:30 PM

Survival of Patients with Dementia with Lewy Bodies: A Meta-Analysis of 236 Postmortem Confirmed Cases

G. K. Wenning, K. Seppi, Innsbruck, Austria, K. Jellinger, Vienna, Austria, G. Ransmayr, Innsbruck, Austria, K. R. Chauduri, London, United Kingdom, A. McKee, Boston, MA, M. Verny, Paris, France, S. E. Daniel, London, United Kingdom, W. Poewe, Innsbruck, Austria, I. Litvan, Bethesda, MD

OBJECTIVE: To analyze the survival of patients withn postmortem confirmed dementia with Lewy bodies (DLB).

BACKGROUND: There are no published reports evaluating predictors of survival in DLB.

DESIGN/METHODS: Kaplan Meier survival analysis was performed in 236 postmortem confirmed DLB patients (135 males and 101 females) derived from literature reports and research files of 6 specialized brain banks in Austria, France, United Kingdom, and the United States.

(alcohol-adjusted OR = 0.21; 95% CI 0.12–0.38), and a 60% reduction in risk was observed among those who had smoked less than or equal to 20 pack-years (alcohol-adjusted OR = 0.37; 95% CI 0.16–0.87).

CONCLUSION: Alcohol and tobacco consumption were both inversely associated with the risk of developing PD. Possible interpretations include: 1) a risk-avoidant premorbid personality is more common among PD cases than controls, 2) chemical constituents of alcohol and tobacco may protect against the development of PD, or 3) alcohol and tobacco exert influences on disease risk through their effects on xenobiotic metabolism.

Supported by: National Institute of Neurological Disorders and Stroke, NIH (RO1 NS31964, RO1 NS31964-S1)

S70.002

4:00 PM

Mid-Life Coffee Consumption Is Inversely and Independently Associated with the Subsequent Diagnosis of Parkinson's Disease in Japanese-American Men

Lon White, G. Webster Ross, Helen Petrovitch, David M. Morens, Andrew Grandinetti, Honolulu, HI

OBJECTIVE: Investigate the association of midlife caffeine intake with lower risk for idiopathic Parkinson's disease (IPD) to determine if it is consistent and independent of confounding by smoking or other factors.

BACKGROUND: An inverse (protective) association of mid-life caffeine consumption with the later development of IPD was noted during a prior analysis of dietary vitamin E as a possible protective factor for IPD (Neurol.1996;46:1270).

DESIGN/METHODS: The Honolulu-Asia Aging Study/ Honolulu Heart Program is a longitudinal study of cardiovascular and neurodegenerative diseases among 8006 Japanese-American men born 1900–1919. Cases of IPD among cohort members were identified in two phases: 1) by review of Oahu hospitalization, death, and private neurologist records for cases recognized 1965–1991, and 2) during the 1991–93 reexamination of surviving participants. While both case subsets met rigorous diagnostic criteria, the latter subset also received a standardized neurologic examination. Of 92 IPD cases, 46 received the standardized neurologic examination. The remaining 46 had either died (n = 38) or declined participation. At baseline examination (1965–67) all subjects provided a 24 hour dietary diary and answered questions on usual diet, coffee, smoking, alcohol, occupation, education, and other factors. Similar questions were asked again at a 1971–74 exam.

RESULTS: Caffeine intake, estimated at the 1965–67 examination using data from a 24 hour diet diary, was almost entirely attributable to coffee. Coffee intake at the 1971–74 examination (as cups in the prior week) was moderately correlated with both the 1965 caffeine (r = 0.50) and the 1965 usual consumption variables (r = 0.41). Lifetime smoking was measured categorically (never/ever/current) and as cumulative cigarette pack years at the 1965–67 and 1972–75 exams. Smoking was significantly correlated with coffee drinking, and both were inversely associated with subsequent IPD. Of variables examined to date, only age, smoking, coffee consumption, and midlife serum uric acid levels have been significantly and consistently associated with the subsequent diagnosis of IPD. Only lifetime smoking represents a possible confounding factor of the influence of coffee. Analyses utilized proportional hazards and multiple logistic regression ami- dlife uent the reference group (low or no coffee drinking) to 0.33 (95% CI 0.19–0.58, $p < 0.0001$) in men who reported a usual consumption of 3 cups of coffee daily in 1965. The independent influence of coffee/caffeine was demonstrated by stratification by smoking, and by comparing strengths of association with and without controlling for smoking. The protective effect was significant in men who had never smoked. Among past or current smokers the protective influence of coffee was only minimally diminished by the inclusion of packyears of smoking and age as controlling covariates. The average interval between the 1965–67 estimate of coffee consumption and the diagnosis of IPD was 14.9 years (range 2–27). Associations between coffee and subsequent IPD, while still significant, were weaker with the 1971–74 indicator.

CONCLUSION: Midlife coffee consumption, like smoking, appears to be a significant, independent, protective factor for the subsequent diagnosis of IPD. While the association appears quite robust in this population, the underlying mechanism is unclear.

Supported by: Department of Defense Grant #DAMD17-98-1-8621, National Institute on Aging contract #N01-AG-4-2149, and National Heart Lung and Blood Institute contract #N01-HC-05102

S70.003

4:15 PM

Lack of Association of Midlife Smoking or Coffee Consumption with Presence of Lewy Bodies in the Locus Ceruleus or Substantia Nigra at Autopsy

G. Webster Ross, Lon R. White, Helen Petrovitch, Honolulu, HI, Daron G. Davis, Lexington, KY, John Hardman, Honolulu, HI, James Nelson, New Orleans, LA, William Markesbery, Lexington, KY, David M. Morens, Andrew Grandinetti, Honolulu, HI

OBJECTIVE: To determine the association of Lewy bodies (LB) in the substantia nigra (SN) or locus ceruleus (LC) at autopsy with cigarette smoking and coffee consumption during midlife.

BACKGROUND: In the Honolulu Heart Program cohort we demonstrated that lifetime cigarette smoking and midlife coffee consumption have independent inverse associations with the development of idiopathic Parkinson's disease. Others have consistently shown that individuals who have smoked cigarettes have a lower risk of developing Parkinson's disease. The cause for this effect has not been determined. If smoking or coffee drinking alter the processes leading to Parkinson's disease then it would be expected that they would protect against the development of Lewy bodies in the brainstem pigmented nuclei. This assumes that Lewy bodies are an accurate indicator of the disease process.

DESIGN/METHODS: The brains of 220 Japanese-American men (mean age at death = 84 years, range 73–97 years) who were participants in the longitudinal Honolulu Heart Program/Honolulu–Asia Aging Study were examined for the presence of Lewy bodies in two brainstem pigmented nuclei (BPN), the substantia nigra (SN) and the locus ceruleus (LC), with hematoxylin - eosin stains. Measures of smoking and coffee consumption were assessed prospectively by interview at Honolulu Heart Program examinations

in 1965–67 (exam 1) and 1971–74 (exam 3). Logistic regression methods were used to examine the associations of smoking and coffee consumption with Lewy bodies in the BPN (either LC or SN) or SN alone, controlling for age.

RESULTS: Among the 218 brains examined, 38 had Lewy bodies in either the LC or SN, 17 had LB in both areas, 17 in the LC only, and 4 in the SN only. None of the 38 individuals with LB had clinical IPD. There was a significant association of LB in the BPN with age (Odds Ratio (OR), ten-year age interval = 2.04, 95% Confidence Interval (CI) = 1.07–3.89). No significant association was found for pack years of smoking at exam 1 (OR = 0.99, 95% CI = 0.98–1.01), or exam 3 (OR = 1.00, 95% CI = 0.99–1.01). Similarly, there was no association found for current, ever, or never smoking with Lewy bodies in the BPN at exam 1 (OR = 0.93, 95% CI = 0.61–1.41), or exam 3 (OR = 1.05, 95% CI = 0.66–1.66). There was no significant association between coffee consumption at exam 1 and the presence of Lewy bodies in the BPN at exam 1 (OR = 0.88, 95% CI = 0.65–1.19) or exam 3 (OR = 0.97, 95% CI = 0.93–1.01). Identical analyses using Lewy bodies in the SN as the outcome yielded similarly negative results.

CONCLUSION: We observed a doubling in the prevalence of Lewy bodies with each decade of advancing age. The inverse association between cigarette smoking and Parkinson's disease does not appear to be related to the formation of Lewy bodies in the substantia nigra, the requisite pathologic lesion in Parkinson's disease. Smoking and coffee drinking may delay or mask the clinical expression of Parkinson's disease through a pharmacologic effect. Alternatively, they may be markers for an undetermined factor that influences susceptibility to the disease.

Supported by: Department of Defense Grant # DAMD17-98-1-8621, National Institutes of Health, National Institute on Aging contract # N01-AG-4–2149, and National Heart, Lung, and Blood Institute contract # N01-HC-05102

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Skin Pigmentation and Risk of Parkinson's Disease (PD)

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OBJECTIVE: To test the hypothesis that greater skin pigmentation protects against the development of PD (Lerner MR, Goldman RS, 1987).

BACKGROUND: MPP+, the active form MPTP, binds to neuromelanin, possibly explaining its selective injury of pigmented neurons. Dermal melanin may bind MPTP-like toxicants and prevent their entry into the brain. If this is true, darker skin color should be inversely associated with PD.

DESIGN/METHODS: Skin pigmentation was assessed objectively using a Minolta Chroma Meter CR-300 and subjectively using a previously-validated self-report instrument in 509 newly diagnosed PD and 541 age- and gender-matched controls, all members of Kaiser Permanente, Northern California. Data were analyzed by logistic regression adjusted for ethnicity, age and gender overall, and for age and gender within ethnicities.

RESULTS: Darker skin color (higher yellow/blue reflectance) was inversely associated with PD overall (adjusted odds ratio (OR): 0.55; 95% CI: 0.36–0.84; p = 0.005) and in Caucasians (OR: 0.46; 95% CI: 0.28–0.78; p = 0.003). In African-Americans, darker skin color (higher red/gree tance) was inversely associated with PD (OR: 0.60; 0.38–0.94; p = 0.027). Self-reported tendency to tan v exposure was inversely associated with PD overall ((95% CI 0.47–1.0; p = 0.05) and in Caucasians (OR: 0 CI: 0.44–1.03; p = 0.066). Lighter skin color (L*) was ated with an increased risk of PD overall (OR: 1.62; 1.05–2.5; p = 0.03), and in African-Americans (OR: 1 CI: 1.03–1.28; p = 0.013).

CONCLUSION: In this multiethnic population, skin color is associated with a lower risk of Par disease. This effect is seen within Caucasians and Americans when analyzed separately. Both objecti surements and self-reported tendency to tan follow t pattern. While other explanations must be considere findings are consistent with the hypothesis that derm nin protects against Parkinson's disease.

Supported by: NINDS RO1 NS31964–01A1

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The Population Prevalence of Atypical Parkinsonian Disorders

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OBJECTIVE: To determine the population preval atypical parkinsonian disorders

BACKGROUND: Atypical parkinsonian disorders multiple system atrophy (MSA) and progressive supra palsy (PSP) are underdiagnosed. One small previous tion study on the prevalence of these relatively rare di has been performed to date (Trenkwalder et al., 1995)

DESIGN/METHODS: The computerised records general practices (GP) in London and Kent, UK, cov population of 121,608 people were screened for all p with a mention of "Parkinson's disease," "parkins "tremor" (with onset after age 50), "progressive supra palsy," "striatonigral degeneration," "Shy-Drager sync "parkinsonism with orthostatic hypotension," and "ot trapyramidal disorders, not otherwise specified," o who had ever received antiparkinsonian medication. A ble patients, who agreed to participate, were seen at h their GP's surgery or at the National Hospital, and recordings were made. The diagnosis of each parkir disorder was made according to the respective pu criteria. All patients with parkinsonism were followe 3-monthly questionnaires and patients with atypical f were reviewed after a period of one year.

RESULTS: The participation rate was 84%. The lence rate for MSA was 3.3 (95% confidence intervals 8.4) per 100,000 (2 probable and 2 possible cases) a PSP 4.9 (95% confidence intervals 1.8 to 10.7) per 100 probable and 1 possible case). Neither of these diagnos previously been made. An additional 4 atypical patie not fulfil published criteria for MSA or PSP.

CONCLUSION: These results suggest that the prev of MSA and PSP is higher than previously estimated, a many patients in the community remain undiagnosed.

Supported by: grant from SmithKline Beecham P ceuticals

APPENDIX K

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Cerebrovascular Pathology and Dementia in Autopsied Honolulu-Asia Aging Study Participants

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ABSTRACT: **Clinicopathologic data from 285 autopsies were analyzed. The decedents were long-standing participants in the Honolulu-Asia Aging Study, a prospective epidemiologic investigation of stroke, neurodegenerative diseases, and aging. We assessed the prevalence at death of four primary neuropathologic processes using specific microscopic lesions as indicators. An algorithm was developed to assign each decedent to one of six subsets, corresponding to pathologic dominance by microvascular lesions (14% of decedents), Alzheimer lesions (12%), hippocampal sclerosis (5%), cortical Lewy bodies (5%), codominance by two or more primary processes (9%), or without a dominant pathologic process recognized (55%). Definite or probable dementia had been identified in 118 of the decedents. The proportions of men in each subset identified as demented were (in the same order) 57%, 53%, 79%, 57%, 76%, and 25%. In this autopsied panel of older Japanese-American men, the importance of microvascular lesions as a likely explanation for dementia was nearly equal to that of Alzheimer lesions. The cerebrovascular lesion type most essentially and inclusively related to dementia was multiple microinfarction.**

KEYWORDS: **dementia; cognitive impairment; aging; pathology; microinfarct; brain; Alzheimer; vascular dementia**

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Ann. N.Y. Acad. Sci. 977: 1–15 (2002). **© 2002 New York Academy of Sciences.**

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INTRODUCTION

Classification of dementia cases ordinarily reflects the underlying structural pathology to which the illness is attributed. During life, the underlying process is inferred from the clinical history and course, neurologic examination, neuropsychologic evaluation, laboratory test results, and neuroimaging. When autopsy findings become available, they provide the ultimate measures by which the causal processes are defined. We conventionally use the pathologist's observations to arrive at a conclusion that either confirms the clinical diagnosis or indicates a different etiology. Recent reports concerning the spectrum of clinical and neuropathologic findings in demented and nondemented persons have emphasized the complexity of these issues as they relate to the common dementing conditions and processes of late life.[1–3]

Our study utilizes the population and prospectively accrued data of the Honolulu Heart Program (HHP) and the Honolulu-Asia Aging Study (HAAS).[4,5] A large body of highly standardized, electronically archived information is available for study participants related to cardiovascular risk factors, physiologic assessments, and sensory and motor functioning. Cognitive function assessments and standardized dementia evaluations for persons with low or marginal cognitive test scores have been administered as part of four examination cycles carried out since 1991. The HAAS research protocol for brain autopsy was established in 1991, and autopsy rates have risen since that time from about 1% to approximately 20% for deaths in study participants. Except for higher rates among demented participants, autopsied decedents are generally representative of all cohort decedents. Most importantly, demented decedents on whom autopsies were completed were not substantially different from nonautopsied demented decedents. Similarly, nondemented decedents who came to autopsy were not substantially different from nondemented decedents who did not.

The HAAS neuropathologist conducting the initial gross brain evaluation is provided with limited clinical information to address immediate questions concerning the cause of death. Subsequent microscopic readings are conducted by a different neuropathologist who has access to information from the gross examination (such as number and location of infarcts, ventricular dilatation, etc.), but is shielded from all clinical information. The autopsy protocol is intended to identify, characterize, and record structural features relevant to brain aging and dementia. Standardized observations are recorded and coded in numeric format for computerized analysis. A central goal of the autopsy protocol is to obtain fully comparable quantitative gross and microscopic observations on all subjects. The neuropathologic examinations are not undertaken with intent to explain a clinical presentation, but to provide similar information on all decedents, regardless of clinical manifestations.

This study design allows an unbiased, naïve search for patterns of association between structural brain changes found at autopsy and the occurrence of dementia in the final years of life. In this report, we describe initial results from such a search, with a special focus on focal ischemic cerebrovascular lesions.

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MATERIALS AND METHODS

The Study Population

The subjects included in this study were decedent members of the Honolulu Heart Program (HHP) and Honolulu-Asia Aging Study cohort (HAAS) who came to autopsy between May 1991 and September 1999. The HHP was established in 1965 with the examination of 8006 subjects, representing approximately two-thirds of the Japanese-American men who were living on Oahu and who had been born in the years 1900 through 1919. Additional examination cycles were conducted in 1967–69 and 1971–73.[5] The first cognitive testing was done at the 1991–93 examination, with the establishment of the HAAS as an offshoot project.[4] Each subsequent cycle of HAAS examinations (1994–96, 1997–98, 1999–2000, 2001–current) has included assessments of cognition and evaluations for dementia.[6] Human Subjects Institutional Review Boards provided approvals, and informed consents were obtained from the participants and/or family members at every examination.

Assessment of Dementia and Definition of Cognitive Impairment

The Cognitive Abilities Screening Instrument (CASI) was administered at least once at every examination cycle. It is a brief, comprehensive test developed specifically for use in cross-national epidemiologic studies of dementia.[7] Possible scores range from 0 to 100. In the HAAS population, a score of 74 corresponds to an MMSE score of 20–21 and was used as the screening score for initiating a full dementia evaluation. A CASI score of 65 corresponds to an MMSE score of 17–18. Scores between 65 and 74 represent mild to moderate cognitive impairment. Scores below 65 are referred to in this report as indicating definite cognitive impairment. Although CASI scores below 65 are usually associated with dementia, poor test performance is occasionally attributed to conditions such as aphasia, deafness, or blindness. Testing was done in English or Japanese, depending on the participant's preference.

Two- or three-stage dementia evaluations were conducted on 10–20% of the HAAS participants at each examination cycle.[4,6] Among the 285 autopsied decedents included in the present analysis, 100 had met DSMIII-R criteria for dementia (classified as definitely demented). An additional 33 received a final CASI score of less than 65. This group included 9 on whom a full dementia evaluation was not completed, while another 9 who were evaluated for dementia received CDR scores of 0.5 and met Cummings-Benson criteria for dementia, but failed to meet DSMIII-R criteria for dementia.[4] These last-mentioned 18 subjects were classified as probably demented. The remaining 15 participants were fully evaluated, but failed to meet either DSMIII-R or Cummings-Benson criteria. These subjects were classified as impaired, but not demented.

Autopsy Recruitment and Permission

Autopsies were discussed with all examined participants at the 1991–93 and 1994–96 examinations. Study participants who had been diagnosed as demented were targeted for special attention, including frequent contacts with family members and provision of information of utility to caregivers. These contacts resulted in higher autopsy rates among demented participants that were unrelated to the type of

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dementia diagnosed. Full informed and witnessed autopsy consents were obtained in all cases.

Autopsy Methods

The autopsy panel described here consisted of 285 HHP and HAAS decedents dying between May 1992 and September 2000. They ranged in age from 74 to 97 years, with a mean age of 84.9 years. The median interval between the final CASI testing and death was 622 days, with 3.1% being less than 90 days and 94% being less than 4 years.

Most autopsies were conducted at 6–48 hours after death. Initial weighing and examination of the external brain occurred at that time, following which samples were removed for biochemical analysis and the brain and upper cord were fixed by submerging in neutral formalin for at least 2 weeks. The brain was then reweighed and the cerebrum examined using 1-cm coronal sections, with 4- to 10-mm sectioning of the midbrain, brain stem, cerebellum, medulla, and upper cord. Detailed, standardized observations were recorded related to all abnormalities, size and architecture of ventricles and major structures, vascular abnormalities, meningeal changes, large infarcts, lacunes, hemorrhages, hematomas, etc.

Blocks of tissue from 38 brain areas were processed, embedded, and sectioned. Routine stains included hematoxylin and eosin (H&E), a modified Bielschowsky, Gallyas, and two immunohistochemical stains [one for the detection of A-β-amyloid (donated by Athena Labs) and the other against α-synuclein]. Microscopic readings were done by one of three neuropathologists after training sessions to ensure comparability. A standard protocol was used for all microscopic examinations, with numerically coded items employed to indicate lesion numbers and characteristics.

Data from the gross and microscopic examinations were converted to electronic format using the Viking data entry system and then to SAS data sets for statistical analysis after incorporation of information obtained during life.

RESULTS

Analyses were undertaken with an expectation that the most common causes of cognitive impairment and dementia were likely to be the Alzheimer process, ischemic cerebrovascular disease, the process underlying the occurrence of cortical Lewy bodies, and hippocampal sclerosis. An initial goal was to develop a summary indicator for each of these primary pathologic processes that would fairly and simply reflect its occurrence in each autopsied individual. Our intention was to develop metrics for these summary indicators based on their strengths of association with poor cognitive test performance. For each primary process, this effort began with an examination of the primary variables thought to reflect the process. The primary variables were individually examined using stratification, graphing, and sequential linear regression methods to identify those that demonstrated the strongest and most inclusive associations with poor scores on the last cognitive tests administered before death. Secondary indicators were created by summing or averaging the values of primary variables measuring the same characteristic (as number or density of specific lesions) across related brain regions. The essential primary or secondary

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variables thus identified were considered cardinal indicators of the process. The third step involved a determination of criterion levels for the cardinal indicators, defining high, intermediate, and negligible associations with dementia or definite cognitive impairment. When a primary process required consideration of more than one cardinal indicator, a system was developed for summarizing them as a single indicator.

Primary variables describing ischemic cerebrovascular disease were defined at the gross and microscopic examinations. Numerically coded descriptions of the number, location (side and region), age (recent vs. remote), and size of all large (volume $\geq$ 1 mL) and small (<1 mL) infarcts and hemorrhages were available from the gross examination. All small infarcts identified on gross examination (almost always apparent because of a cystic loss of central tissue) were designated lacunes. Secondary variables related to large infarcts and hemorrhages included the total number of temporally remote large lesions on the left, right, and both sides collectively. Secondary variables related to lacunes included the total number and the number after excluding cerebellar lacunes.

Microinfarcts were identified in sections from the left and right caudate nucleus, putamen, globus pallidus, thalamus, internal capsule, frontal lobe, temporal lobe, parietal lobe, occipital lobe, hippocampus, and cerebellum. For purposes of this analysis, we have defined a microinfarct as a focal lesion attributed to ischemia, found only on microscopic examination, and judged to be temporally remote. In practice, such lesions were identified as foci of pallor, neuronal loss, and gliosis found on microscopic examination and unrelated to infarcts identified during the course of the gross examination. Cystic microinfarcts (sometimes referred to as microlacunes) tended to be somewhat larger than noncystic microinfarcts. Noncystic microinfarcts typically had a diameter in the range of 50–400 microns. Since microinfarcts were identified on 8-micron, H&E-stained tissue sections, their 2-D structure did not allow an assessment of the lesion's true size and 3-D architecture. Although most of the cortical microinfarcts were located toward the base of the gray matter, a few were actually microlacunes located in white matter just beneath the gray cortical ribbon. Focal leukoencephalopathic lesions were also considered a type of microinfarct, but were substantially less common than white matter microlacunes or microinfarcts in gray matter. Secondary variables related to microscopic ischemic lesions included the total number of microinfarcts noted in cortical sections (4 lobes, left and right, 1 section reviewed from each site) and in the basal ganglia (left and right, typically 2 slides) and thalamus (left and right, typically 2 slides).

Among the 285 autopsies, 18% had one large infarct. An additional 12% had more than one large infarct. Six percent had one large hemorrhage and 0.4% had more than one. One small hemorrhage was found in 2.3% and more than one in 1.1% of autopsies. One decedent had a very large cerebral cyst that appeared to have developed as a result of a hemorrhagic infarct. One lacune was found in 19%, two in 9.5%, and three or more in 17%. Although lacunes were more common in persons who had large infarcts, a substantial number of decedents had either one or the other. While microinfarcts were more often observed in individuals who had lacunes and/or large infarcts, they also occurred in persons without the larger lesions.

After exploratory analyses, we identified two secondary variables, both reflecting microscopic infarcts, that were collectively sufficient to capture nearly all of the apparent influence on cognitive test performance attributable to focal ischemic

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TABLE 1. Codistribution of high, intermediate, and negligible numbers of microinfarcts observed in H&E sections of neocortex, basal ganglia, and thalamus

Basal ganglia and thalamus microinfarcts	Neocortical microinfarcts		
	<3	3–4	≥5
<3	83/230 (36%)	6/10 (60%)	6/10 (60%)
3	3/8 (38%)	1/1 (100%)	5/5 (100%)
≥4	10/15 (67%)	2/3 (67%)	2/3 (67%)

NOTE: Data show the proportions of demented (definitely or probably) persons/total in whom the lesions were observed.

cerebrovascular disease. These were (1) the number of temporally remote microinfarcts identified in the neocortex (from review of 8 slides, 1 each from the left and right neocortical lobes) and (2) the number of temporally remote microinfarcts identified on review of sections from the basal ganglia and nearby structures (left and right caudate, putamen, internal capsule, globus pallidus, and thalamus; usually on review of 2 or 4 H&E-stained slides). When these were taken into account, neither large infarcts, lacunes, nor hemorrhages added significantly to the variance in the final cognitive function test scores; that is, only the two microinfarct variables were independently associated with poor CASI test scores. The individual strengths of association of the two cardinal indicators with the last available CASI score were then examined to designate high, intermediate, and low/negligible levels. A total of 5 or more neocortical microinfarcts was designated as representing a high level of cortical microvascular disease, while 3 and 4 were considered intermediate. For lesions observed in the basal ganglia and thalamus, 4 or more microinfarcts was designated as high and 3 as intermediate. Finally, a microvascular summary factor was defined in which (1) a high level of one or both of the cardinal indicators was observed, (2) an intermediate level was recorded for one or both, or (3) a negligible level existed when neither of the cardinal microvascular lesion indicators reached an intermediate level. TABLE 1 provides a description of the frequency with which microinfarcts were observed in these two brain regions in the total autopsy panel (denominator of the fraction shown) and among demented decedents (the numerator). These data indicate that negligible levels of microinfarcts in both brain regions were observed in 230 of the 285 decedents, while 36 had high levels of these lesions in one or both brain regions. Among decedents in whom high levels were found in neither region, 19 had intermediate levels or microinfarcts in one or both regions. Although persons with neocortical microinfarcts were somewhat more likely to have microinfarcts in the basal ganglia and/or thalamus than were individuals without cortical microvascular lesions, a substantial proportion of decedents had these lesions at one site, but not the other. Further, lesions at either site appeared to be associated with dementia or cognitive impairment, as shown in TABLE 1.

In the case of the Alzheimer process, the primary variables available for examination included diffuse plaque (DP), neuritic plaque (NP), and neurofibrillary tangle (NFT) counts (per mm^2 field, counting 5 fields per region) in the following regions: left frontal, left parietal, left temporal, left occipital, left CA-1, left subiculum, left entorhinal cortex, and left amygdala. For each region, we considered both the

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TABLE 2. Codistribution of high, intermediate, and negligible levels of neuritic plaques (highest count among 20 fields counted) and high, intermediate, and negligible levels of neurofibrillary tangles (average count over 20 fields counted) in the 4 neocortical lobes

Neocortical neurofibrillary tangles	Neocortical neuritic plaques		
	<3	3–16	>16
<2	61/174 (35%)	8/24 (33%)	1/5 (20%)
2–12	6/21 (29%)	18/32 (56%)	5/7 (71%)
>12	0/1 (0%)	10/12 (83%)	9/9 (100%)

NOTE: Fractions are presented for occurrence in demented (definite or probable)/total decedents.

maximum count observed in any single field and the average count across all available fields (usually 5 per region). We also considered the following secondary variables: average and maximum counts in the neocortex (4 lobes), hippocampus (CA-1, left subiculum), and limbic structures (CA-1, subiculum, entorhinal cortex, amygdala). We were unable to examine the Braak stage as a summary indicator because these results were incomplete at the time of the analyses presented here.

Exploratory analyses indicated that two of the secondary variables were collectively sufficient to capture nearly all of the influence attributable to the Alzheimer process. These were (1) the maximum NP count observed in any of the 20 neocortical fields examined and (2) the average NFT count across the 20 neocortical fields. Neither NFT densities in the hippocampus nor other limbic structures were independently associated with poor CASI test performance once cortical lesion densities were considered. Hippocampal NP densities occasionally demonstrated a marginally significant, independent association with poor cognitive test scores in certain subsets of the population, but were not a major factor. The two cardinal indicators of the Alzheimer process were then examined further to designate high, intermediate, and low/negligible levels according to their associations with dementia or definite cognitive impairment. Maximum neocortical NP counts of >16 were considered high, 3–16 intermediate, and <3 (per mm^2) low or negligible. Corresponding levels for average neocortical NFT counts (4 lobes, 20 fields) were >12, 2–12, and <2 per mm^2. The Alzheimer summary factor was defined as high when a high level was observed for either of the cardinal indicators, as intermediate when both of the cardinal indicators reached intermediate counts, and as negligible if neither or only one reached intermediate levels. Negligible levels of NP and NFT were observed in 174 of the 285 autopsied decedents and in 61 of those who had been demented or impaired. High levels of both lesion types were observed in 9 cases, all of whom had been demented or impaired. Intermediate levels of NP and intermediate levels of NFT were found in 32 decedents, 18 of whom had been demented or impaired. Data are shown in TABLE 2.

For the Lewy body process, the primary variables available for all autopsies were the number of Lewy bodies observed on single H&E-stained sections through the substantia nigra and the locus ceruleus. When one or more Lewy bodies were found in either of the pigmented brain stem nuclei, additional counts of Lewy bodies were

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TABLE 3. Autopsied decedents subdivided according to levels of the primary pathological lesion types

	Number		Percent	
	Decedents with	Def/prob demented	Affected (dem/with)	Prevalence (with/285)
Microvascular lesions; summary indicator				
Negligible microvascular lesions; both cardinal indicators low	230	83	36%	80%
Intermediate microvascular lesions; at least 1 indicator intermediate	19	10	53%	7%
High microvascular lesions; at least 1 cardinal indicator high	36	25	69%	13%
Alzheimer lesions; summary indicator				
Negligible Alzheimer process (none, few, or intermediate NP or NFT)	219	75	34%	77%
Intermediate Alzheimer process (intermediate NP and intermediate NFT)	32	18	56%	11%
High Alzheimer process (high NP and/or high NFT)	34	25	74%	12%
Hippocampal sclerosis				
Not noted	259	98	38%	91%
Present	26	20	77%	9%
Cortical Lewy bodies				
None, 6 cortical regions	262	104	40%	92%
1 or more cortical Lewy body	23	14	61%	8%

NOTE: Data are the total number of decedents, the number demented, the percent of persons having that level who are demented (affected), and the percent of decedents having the neuropathologic feature indicated (prevalence of the neuropathologic lesion). Data for each lesion type are presented independent of the others.

available for anti-α-synuclein-stained sections from the left anterior cingulate gyrus, left insula, left frontal cortex, left parietal cortex, left temporal cortex, left entorhinal cortex, and left amygdala. Among the 50 decedents in which one or more Lewy bodies were noted in sections of the pigmented brain stem nuclei, 23 were found to have Lewy bodies in one or more of the neocortical regions. When Lewy bodies were limited to the substantia nigra and/or locus ceruleus, no association with dementia or definite cognitive impairment was noted. In contrast, when Lewy bodies were found in any of the neocortical regions, there was a substantial association with dementia or definite cognitive impairment. Based on these observations, we defined a cortical Lewy body summary indicator as high when one or more neocortical Lewy bodies had been observed and as negative in all other cases.

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TABLE 4. Logistic regression analysis of the associations of the summary indicators of the four primary pathogenic processes with definite or probable dementia

Independent variables	Odds ratio	95% CI
Neocortical NP and NFT		
None or negligible	1.0	[reference]
Intermediate	1.98	0.89–4.42
High	4.27	1.78–10.24
Cortical Lewy bodies		
None	1.0	[reference]
At least 1 found	2.17	0.83–5.64
Hippocampal sclerosis		
Not noted	1.0	[reference]
Present	4.96	1.81–13.58
Microvascular ischemic lesions		
None or negligible	1.0	[reference]
Intermediate	2.36	0.87–6.41
High	4.59	2.07–10.19

NOTE: Model also includes years of education and age at death as covariates, in addition to the other variables listed.

For hippocampal sclerosis, the only variable available was the neuropathologist's notation as to whether it was or was not observed in either or both hippocampi. Among the 285 autopsies, hippocampal sclerosis was noted in 26 cases, and a strong, statistically significant association with dementia was apparent. The summary indicator was defined as high when hippocampal sclerosis was observed and as negative when it was not.

Data related to the prevalence of the four primary processes using their summary indicators are shown in TABLE 3. Based on the definitions described, high levels of the Alzheimer and microvascular processes occurred with nearly the same frequency, while an intermediate level of the Alzheimer process was more common than an intermediate level of the microvascular process.

Multivariate logistic regression analysis was then conducted to examine the simultaneous associations of the four summary indicators with definite or probable dementia. The results of this analysis, presented in TABLE 4, indicate significant, independent associations of three of the four pathogenic processes with this endpoint. The association of the cortical Lewy body indicator approached (but did not reach) statistical significance, reflecting the relative infrequency of this lesion and the occurrence of a second lesion type in some of the decedents in whom cortical Lewy bodies were found.

A hierarchical system was devised for assigning each autopsied decedent to one of six exhaustive and mutually exclusive categories, based on the dominance of a single process or the codominance of more than one process. The finding of hippo-

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campal sclerosis or of any cortical Lewy bodies was considered to represent a high level of that process. When only a single process was expressed at a high level, it was considered dominant. When either the microvascular process or the Alzheimer process occurred at an intermediate level, but none of the other processes were evident, that process was considered dominant. When two or more of the lesion types were present at high levels, they were considered codominant. When both microvascular and Alzheimer lesions were noted at an intermediate level and neither hippocampal sclerosis nor cortical Lewy bodies were noted, they (the microvascular and Alzheimer processes) were identified as codominant. Using this system, all 285 decedents (including the 118 who had been definitely or probably demented) were assigned to one of the six possible categories, corresponding to dominance by one of the four primary processes, to codominance of two or more processes, or to none or a negligible level of all four primary lesion types. TABLE 5 provides information on the frequency with which the four pathogenic processes occurred alone or in combination, on the average CASI score achieved on final testing prior to death, and on the prevalence of dementia or definite cognitive impairment in the six subsets of the autopsy panel.

Among 36 decedents in whom high levels of microvascular lesions were observed, one-quarter occurred in conjunction with one or more codominant lesions: 5 with high levels of the Alzheimer process (including 1 with Lewy bodies as well), 2 with cortical Lewy bodies, and 2 with hippocampal sclerosis. Among the decedents with codominant lesions, 89% (8/9) had been demented or cognitively impaired. Among the 27 with high levels of microvascular lesions and no codominant process, 70% had been demented or cognitively impaired.

Of the 34 persons in whom high levels of the Alzheimer process were found, half occurred with one or more codominant lesions: 5 with high levels of microvascular lesions (1 with Lewy bodies as well, as mentioned in the paragraph above), 9 with hippocampal sclerosis (including 2 with cortical Lewy bodies as well), and 3 with cortical Lewy bodies as the only codominant lesion. Dementia or cognitive impairment had been noted in 94% (16/17) of the decedents in whom high levels of Alzheimer lesions occurred in conjunction with one or more codominant lesion types. Among the 17 decedents in whom no other codominant process was apparent, 65% (11/17) had been affected.

Codominant lesions were common among decedents with hippocampal sclerosis and with cortical Lewy bodies. When codominant lesions were noted, the proportion who had been demented or cognitively impaired appeared to be somewhat increased, compared to instances when hippocampal sclerosis or cortical Lewy bodies were the sole or dominant lesion type.

Among the 118 decedents who had been identified as definitely or probably demented, there were 39 in the "none of the above" category (TABLE 5), based on a paucity of lesions related to the four primary pathologic processes addressed in this analysis. Included in this group was 1 person whose CASI score was well within the normal range, but whose behavior and clinical history had suggested a dementing illness. Another subject (diagnosed as having a dementia of unknown type) had a low normal CASI score and declined only 9 points over a 3-year follow-up period. One other subject (who had been classified as VaD during life) was found at autopsy to have a large cavitary lesion probably related to a remote hemorrhagic infarct. Five

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TABLE 5. Division of 285 autopsied HAAS decedents into exhaustive and mutually exclusive subsets based on neuropathologic lesion patterns

Vascular	Alzheimer	Hipp sclero	Cx Lewy	Last CASI	*N*	Demented
(1) Sole or dominant lesion = microinfarcts						
High	Low/none	None	None	44	21	13 (62%)
Intermediate	Low/none	None	None	56	13	6 (46%)
High	Intermediate	None	None	36	6	4 (67%)
Total				46	40	23 (57.5%)
(2) Sole or dominant lesion = Alzheimer						
Low/none	High	None	None	45	17	11 (65%)
Low/none	Intermediate	None	None	52	17	7 (41%)
Intermediate	High	None	None	–	0	–
Total				48	34	18 (53%)
(3) Sole or dominant lesion = hippocampal sclerosis						
Low/none	Low/none	Present	None	39	9	6 (67%)
Intermediate	Low/none	Present	None	8	2	2 (100%)
Low/none	Intermediate	Present	None	37	3	3 (100%)
Total				34	14	11 (79%)
(4) Sole or dominant lesion = cortical Lewy bodies						
Low/none	Low/none	None	Present	65	11	5 (45%)
Intermediate	Low/none	None	Present	–	0	–
Low/none	Intermediate	None	Present	42	3	3 (100%)
Total				51	14	8 (57%)
(5) Codominant (mixed) lesions						
Intermediate	Intermediate	None	None	70	3	1 (33%)
High	High	None	None	25	4	4 (100%)
High	High	None	Present	70	1	0 (0%)
High	Interm/low/none	Present	None	51	2	2 (100%)
High	Interm/low/none	None	Present	17	2	2 (100%)
Interm/low/none	High	Present	None	23	7	6 (86%)
Interm/low/none	High	None	Present	11	3	3 (100%)
Interm/low/none	High	Present	Present	35	2	1 (50%)
Interm/low/none	Interm/low/none	Present	Present	90	1	0 (0%)
Total				35	25	19 (76%)
(6) None of the above						
Low/none	Low/none	None	None	72	158	39 (25%)
TOTAL					285	118 (41%)

NOTE: Most numbers represent the actual number of decedents. The value for last CASI is the mean of the scores for the individuals in that cell.

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subjects in this group were noted to have prominent extrapyramidal signs; in 2 of these subjects, Lewy bodies were found in both the substantia nigra and the locus ceruleus, with none found in 6 neocortical areas. Two subjects had quite low CASI scores (54 and 45), but did not complete a dementia evaluation. There were 4 other subjects in whom dementia was clearly present, but the clinical presentation was sufficiently unusual so that it was considered to be of unknown cause. The remaining 25 decedents in this group had been diagnosed during life as having Alzheimer's disease and/or vascular dementia.

The interval between the date of autopsy and that of the last available cognitive test score was related both to the neuropathologic indicator variables and to the final test score. In general, individuals in whom the interval was 4 years or longer tended to show a lower prevalence at death of lesions and better final test scores. Among decedents in whom substantial lesions were apparent at autopsy, those in whom the interval was shorter tended to have been more impaired on their final test performance. This appeared to be true for all four of the primary pathological processes, although small numbers preclude confidence in the validity of this impression. A similar relationship was noted among decedents in whom few or no microscopic lesions of the types sought were observed; that is, in this group, as in the groups with substantial brain lesions, a shorter interval between the final cognitive test administration and death was strongly associated with worse performance on the test. Among the 118 decedents who had been demented at last testing, the interval between testing and death was less than 3 years in 92.5% and less than 4 years in 98.5%. Among the 152 cognitively unimpaired decedents, the interval was less than 3 years in 84% and less than 4 years in 89.5%. Since we cannot know the prevalence and density of neuropathological lesions in persons who did not die, the reasonable expectation that persons with such lesions would be at a higher risk for death cannot be inferred with certainty. Similar difficulties preclude conclusions regarding the natural history of the disease process as it proceeds from neural insult, to structural lesion, to dementia, and then to death.

COMMENT

In this report, we have presented an algorithmic method for attribution of dementia or cognitive impairment to one or a mixture of four relatively common pathogenic processes based on key neuropathologic lesions identified at autopsy. For the Alzheimer process, our definitions of high and intermediate neocortical densities of NPs and NFTs approximate those required by the CERAD neuropathologic criteria for definite and probable AD.[8] For hippocampal sclerosis and cortical Lewy bodies, the simple recognition of the lesions at autopsy was associated with a high prevalence of dementia in the months or years prior to death. For ischemic cerebrovascular disease, our algorithm employed only two indicators, both reflecting the numbers of microinfarcts observed on microscopic examination of brain tissues.

Among several pathologic indicators of ischemic cerebrovascular disease examined, only two were inclusively essential correlates of dementia. They were (1) the number of microinfarcts found in cortical tissues on review of 1 slide each from the 8 left and right neocortical lobes and (2) the number of microinfarcts in basal ganglia and thalamus, typically identified on review of 2–4 H&E-stained slides. The number

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of cases of dementia associated with microvascular pathology was quite similar to the number associated with lesions representing the Alzheimer process. We interpret this as implying that focal microvascular ischemic processes are of major importance in the pathogenesis of vascular dementia in the HAAS cohort. The lack of independent association of large and small (lacunar) infarcts with dementia or cognitive impairment once the microscopic infarcts were considered cannot be taken as proof that they (the grossly apparent infarcts) had no influence on that endpoint, but suggests that microvascular lesions may be the more inclusive and more essential indicators of the usual type of ischemic cerebrovascular disease associated with dementia in this cohort. Although the majority of clinical research studies of vascular dementia have employed definitions that explicitly or implicitly attributed the illness to lacunar infarcts, large vessel infarcts, or white matter changes large or extensive enough to be identified on neuroimaging, others have also emphasized the potential importance of microinfarcts.[2,9,10]

The data presented here indicate that dementing illnesses in older persons may often involve multiple pathogenic processes and that multiple processes may confer an increased probability of dementia. Similar findings have been reported from other studies.[1,11] However, our results fail to ~~support~~ demonstrate a definite synergism between intermediate levels of Alzheimer and microvascular processes as a common phenomenon in the development of dementia.

In this series of autopsies, there were 26 instances in which the pathologist noted hippocampal sclerosis. A similar proportion has been reported from one other survey of brain changes at autopsy in an older, community-based cohort.[3] Although our data suggest that some of these may be associated with the Alzheimer process, it appears that a substantial proportion (more than half) may be unassociated with infarcts or the Alzheimer process.

An unexpected aspect of the data presented in TABLE 5 is the high proportion (33%) of demented subjects in whom the condition could not be attributed to any of the four primary pathogenic processes represented in the algorithm or to a combination of them. In more than three-quarters of these, the dementia had been attributed during life to an unknown cause or to AD and/or VaD. It is widely believed that, with autopsy, most cases of dementia can be confidently linked to a specific disease process. Nonetheless, prior estimates of the proportion of late-life dementia cases in which the condition cannot be attributed to a specific histopathologic lesion or disease process have been in the range of 10–50%.[2,12–14] Our figure may be higher than often reported in autopsied cases of patients with AD or VaD because of the older age of the HAAS cohort and because they were found in the course of a population survey rather than being referred for evaluation of a recognized illness. Nevertheless, it is remarkable that this single subset included a substantially higher proportion of the demented cohort members than any of the five process-specific subsets. A few of these cases may have been due to nondementia conditions, such as aphasia, blindness, depression, etc. In addition, some part of the finding may be attributed to inadequate measurement of one or more of the primary pathogenic processes by the indicators used or to some other artifact generated by the analytic system. It is possible that the algorithmic method used here fails to correctly categorize some cases because the numerically codified data currently available may incompletely reflect certain neuropathological subtleties and that a more thorough evalua-

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tion of individual cases will allow unambiguous objective classification in many instances. Examples of structural abnormalities not taken into account in the algorithmic classification described here include (1) occasional very large infarcts or hemorrhages (these will explain at least 1–3 cases), (2) hemispheric asymmetry of disease, with extensive Alzheimer or Lewy body lesions on the opposite (right) side of the brain (Bielschowsky- and anti-α-synuclein-stained slides were examined from only one hemisphere), (3) general or regional atrophy, with loss of neurons or synapses, (4) deep white matter disease, (5) gliosis, (6) amyloid angiopathy, (7) trauma or lesions such as subdural hematoma, (8) other causes of dementia (such as frontotemporal dementia or progressive supranuclear palsy), and (9) other vascular abnormalities (aneurysms, stenosis, etc.).

It is unclear if our findings are broadly applicable or if they are relevant only to older Japanese-ancestry men. It is also unclear if the functional impairments seen in HAAS subjects with focal microvascular pathology were directly due to losses of neurons and synapses in infarcted foci or if the ischemic process implied by the occurrence of microinfarcts has a more generalized influence on the functioning of neurons in surrounding areas. Efforts are under way to assess relationships of microinfarcts with global and regional atrophy and to identify specific risk factors, neuroimage findings, and clinical features associated with high levels of microinfarcts in the neocortex, basal ganglia, and thalamus.

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1538 LOW BLOOD PRESSURE AND RISK OF INCIDENT ALZHEIMER'S DISEASE AND DEMENTIA IN THE KUNGSHOLMEN PROJECT

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Background and Aims: Longitudinal studies have shown that elevated blood pressure increases the risk of Alzheimer's disease (AD) or other dementias. Conversely, a higher prevalence of AD or dementia has been reported in persons with low blood pressure. The inverse association between blood pressure and dementia prevalence is usually interpreted as a consequence of dementia process. This study was aimed at examining whether low blood pressure could predict the risk of incident AD or dementia in the elderly. *Methods:* A community cohort of 1270 dementia-free subjects who were living in the Kungsholmen district of Stockholm and aged 75+ years was longitudinally examined twice over 6 years to detect incident dementia using the DSM-III-R criteria. Data were analysed with Cox proportional hazards models taken into account major potential confounders. *Results:* Over the follow-up period, 339 subjects were diagnosed with dementia (256 of them with AD). Subjects with high systolic pressure (>180 versus 141–180 mm Hg) had an adjusted RR of 1.4 (95% CI 0.9–2.1) for developing AD or 1.5 (95% CI 1.0–2.1, $P = 0.03$) for developing all dementias, but there was no significant association between low systolic pressure (−140 mm Hg) and incidence of dementia. In contrast, low diastolic pressure (−65 versus 66–90 mm Hg) led to an adjusted RR of 1.6 (95% CI 1.1–2.3) for AD or 1.4 (95% CI 1.0–2.0, $P = 0.05$) for all dementias, while high diastolic pressure (>90 mm Hg) was not significantly related to dementia incidence. Such an association was statistically significant particularly in persons who were treated with anti-hypertensive drugs. *Conclusions:* High systolic and low diastolic pressure are recognised to be clinical markers of large arterial stiffness in old people. Thus, the observed association of both high systolic and low diastolic pressure with increased incidence of dementia can be interpreted as an effect of atherosclerosis on the development of AD and dementia.

1539 INCIDENCE RATES OF DEMENTIA, ALZHEIMER'S DISEASE AND VASCULAR DEMENTIA IN THE KAME PROJECT: ROLES OF AGE, GENDER, EDUCATION AND APOLIPOPROTEIN E

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Since 1992, we have followed a cohort of Japanese Americans for incident dementia (The Kame Project). Of 1869 at risk, individuals who scored 87 on the CASI received a full clinical exam. New cases were defined as DSM-IV positive and CDR 1+. We calculated incidence rates using both the person–years and the penalized likelihood methods with age as the time scale. Till 1 July 2001, 163 new cases of dementia were found in 10,376 person–years (PY) (15.7/1000 PY); 80 with Alzheimer's disease (AD) (7.7 per 1000 PY); 35 vascular dementia (VaD) (3.4 per 1000) and 48 other causes (4.6 per 1000) (DSM-IV). Rates increased from 1.7/1000 PY for ages 65–69 to 206.7 in those aged 95+. The hazard ratio (HR) for dementia was 2.5 (95% CI: 1.4–3.7) for apolipoprotein E (ApoE)-ε4 positives compared with those with no ε4, and was stronger in AD (HR = 3.3, 95% CI: 1.2–5.4), but was absent for VaD (HR = 1.3, 95% CI: 0–2.98). There was a slight excess risk of AD among women (HR = 1.69, 95% CI: 0.92–2.51). For all dementias and AD, there was an inverse association between years of education and AD: compared with 13+, the HR for <9 was 6.4 (95% CI: 3.31–9.51); and for 9–12, 2.6 (95% CI: 1.06–4.03). A proportional hazard regression model with gender, education, as well as gender–age, education–age, gender–education and gender–education–age interactions showed that, in the high education group, no significant effect of gender on dementia across the age span was present, but in the low education group, men were at higher risk than women in the early age period and the reverse was true in the late age period. The same finding did not apply in AD. There also was a strong education effect throughout the age period for both genders, with younger, lower educated men (age 65–84) having the highest risk. Our results show strong associations of dementia and AD with age, gender, education and ApoE-ε4, as well as 2.25 times more AD than VaD in this population of older Japanese Americans.

1540 PREVALENCE ESTIMATES FROM A POPULATION-BASED STUDY OF FRONTOTEMPORAL DEMENTIA IN THE NETHERLANDS

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Background: Since 1994, a population-based study of frontotemporal dementia (FTD) in The Netherlands attempts complete ascertainment of FTD, and preliminary prevalence estimates from this study were published in 1998. Further expansion demonstrates a higher prevalence, and enables a better estimation of τ mutation frequency in this FTD population. *Methods:* All neurologists and physicians in nursing homes received a yearly postal enquiry about suspected FTD cases. FTD was diagnosed in 220 patients according to the Lund–Manchester criteria, supported by neuroimaging and neuropsychology. *Results:* Of 220 FTD patients, 156 (71%) were alive and affected on 1 January 1998, resulting in an estimated prevalence of 2.2 per 100,000 in ages 50–60 years, 4.3 per 100,000 in ages 60–70, and 3.6 per 100,000 in ages 70–80. The average age at onset of the 220 patients (54% female) was 57.1 ± 9.0 years. Dementia in one or more first-degree relatives was found in 43% of patients. A mutation screening in exons 9–13 of the τ gene showed 29 (13%) patients with mutations (18 P301L, four G272V, four R406W, one ΔK280, one S320F, one L315R). Of patients with a positive family history for dementia, 36% had a τ mutation. One hundred twenty-seven patients were admitted to a nursing home 4.6 ± 2.4 years after onset, and 93 patients died after a disease duration of 8.0 ± 3.6 years. Of the 41 patients autopsied, neuropathological findings were consistent with the diagnosis dementia lacking distinctive histology in 27 (66%) cases, Pick's disease in eight (21%), and tauopathy related to τ mutation in six (13%). *Conclusions:* The prevalence of FTD has shown to be at least twice as high as previously reported, confirming that FTD is more common than expected. The frequency of τ mutations (13%) remains considerably high within the total FTD population, and justifies mutation screening in FTD patients with a positive family history for dementia.

1541 THE RELATIONSHIP OF LEWY BODIES TO ALZHEIMER LESIONS AND COGNITIVE FUNCTION IN A POPULATION BASED AUTOPSY SERIES

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Objectives: Determine if brainstem or cortical Lewy bodies (LB) are associated with presence or density of neuritic plaques (NPs) or neurofibrillary

tangles (NFTs) in neocortex or hippocampus; regional neuronal loss; lower performance on cognitive testing; and clinical dementia. *Methods:* Autopsies are sought on all participants in the longitudinal Honolulu–Asia Aging Study. H&E stained sections from substantia nigra and locus ceruleus were examined for LB. If present in the brainstem, LB counts were performed on multiple areas of cortex stained for α synuclein. Three mutually exclusive groups, No LB (NLB), LB confined to brainstem (BSLB), and brainstem and cortical LB (CLB) were compared for presence and density of Alzheimer lesions, regional neuronal loss, cognitive test scores, and dementia diagnoses. *Results:* Of 324 brains, 277 had NLB, 9 had BSLB and 38 had CLB. Mean age of the three groups was 85, 86, and 88, respectively. The three groups had similar frequency and mean densities of NP and NFT in the neocortex and hippocampus. Age adjusted odds ratio (OR) for having neocortical NP in the BSLB group was 0.9 (95% CI = 0.23–3.5) and for CLB was 0.83 (95% CI = 0.44–1.6) compared to the NLB group. Findings were similar for neocortical NFT. Cortical LB did predict neuronal loss as judged by the neuropathologist in the nucleus basalis and amygdala. Age adjusted OR for neuronal loss in the nucleus basalis and amygdala for the CLB group compared the NLB group was 3.6 (1.7–7.6) and 2.4 (1.2–4.6), respectively. Although the CLB group had lower age adjusted cognitive scores than the NLB group, this was not statistically significant. In the NLB group 33% were demented, while 50% of those in the BSLB and CLB groups were demented. *Conclusions:* There was no relationship between the presence of LB isolated to the brainstem or cortical LB and presence of either NP or NFT in neocortex or hippocampus. Cortical LB were associated with neuronal loss in the nucleus basalis and amygdala. Cognitive function was lower and dementia diagnoses were more common in those with LB.

1542 PREVALENCE OF DEMENTIA WITH LEWY BODIES IN A GENERAL POPULATION AGED 75 YEARS OR OLDER

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Background: The estimated prevalence rates of dementia with Lewy bodies (DLB), ranging from 15 to 35% of all demented subjects, are based mainly on neuropathological series and on registers of research centres. No population-based prevalence rates of DLB in the elderly have been reported. *Objective:* To estimate the prevalence of DLB according to the consensus criteria in a general population aged 75 years or older. *Methods:* The Kuopio 75+ Study is a population-based health survey focused on clinical epidemiology of dementia and functional capacity among elderly subjects aged 75 years or older. A random sample of 700 subjects was drawn from a total population born before 1 January 1923, living in the city of Kuopio, northeast Finland, on 1 January 1998 ($n = 4518$). The study subjects underwent a structured interview and clinical examination. *Results:* In the Kuopio 75+ Study, 601 elderly subjects (86% of the random sample) were examined. A dementia disorder was diagnosed in 137 subjects, the prevalence being 22.8% (95% CI 19.4–26.2%). The prevalence rate for DLB was 5.0% (95% CI 3.2–6.7%), comprising 22% of all demented subjects. Probable DLB was diagnosed in 20 subjects, 3.3% (95% CI 1.9–4.8%), and possible DLB in 10 subjects, 1.7% (95% CI 0.6–2.7%). The prevalence rate for Alzheimer's disease was 10.6% (47% of all demented subjects), for vascular dementia 5.3% (23%), and for other types of dementia disorders 1.8% (8%). *Conclusions:* In a general population aged 75 years and older, the prevalence rate of the disorder fulfilling the diagnostic criteria of DLB is half that of Alzheimer's disease and the same as for vascular dementia.

Oral Session (T9): Neuroimaging

1543 IMPROVING THE SPEED OF ASSESSMENT OF MAGNETIC RESONANCE IMAGING MEASURES OF THE PROGRESS OF ALZHEIMER'S DISEASE ARE 12-MONTH LONGITUDINAL STUDIES FEASIBLE?

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Objective: We wished to evaluate global and regional rates of brain atrophy on T1-weighted brain MR images for a group of Alzheimer's disease (AD) patients and age-matched normal control (NC) subjects measured over 12 months; this period is a significantly shorter time than most previous studies. This shortened time frame is more appropriate to clinical and drug studies than are more prolonged studies conducted over several years. *Methods:* Using three-dimensional MRI techniques, the spatial resolution was 0.9 mm in all three directions. Three rates of brain atrophy: the rate of atrophy in the cerebrum, the rate of lateral ventricle enlargement and the rate of atrophy in the region of temporal lobes were evaluated. Volumetric analysis was performed with customised methods developed for this project. *Results:* Fourteen AD patients (five with initial MMSE > 20, seven with $10 <$ MMSE < 20, two with MMSE < 10) and 14 age-matched NC subjects (MMSE average 29.6 ± 1.0) were studied for 12 months. The age of the two groups was 66.9 ± 8.6 years for AD patients and 71.5 ± 3.4 years for controls. There were five females and nine males in each group. There was significant overlap between the AD and NCs for cross-sectional measures of atrophy (not shown). The rates of change for these measures were then evaluated and results are shown in the table below. Rates of cerebral volume change are shown in the table below:

	AD (%)	NC (%)	AD/NC ratio
Cerebral atrophy	2.4 (±1.2)	0.4 (±0.5)	6.0
Lateral ventricle enlargement	13.8 (±4.8)	1.9 (±4.2)	7.3
Temporal lobe atrophy	3.4 (±2.0)	0.7 (±0.0)	4.9

By using both the lateral ventricle rate of enlargement and temporal lobe atrophy together, we were able to construct a discriminant function that completely separated the AD group from the NCs. We found no relationship between the rates of lateral ventricle enlargement, temporal atrophy and the changes in the MMSE. We conclude that short time frame longitudinal MRI studies of AD patients can reveal clear cut differences between AD and NC subjects in a 12-month period. These results can be used in power calculations to determine group sizes required to detect various treatment effect sizes.

1544 MAPPING THE EVOLUTION OF REGIONAL ATROPHY IN ALZHEIMER'S DISEASE: UNBIASED ANALYSIS OF FLUID-REGISTERED SERIAL MAGNETIC RESONANCE IMAGES

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Background: Alzheimer's Disease (AD) is characterised by progressive brain atrophy which may be assessed using volumetric MRI. Understanding the

Movement Abnormalities and Incidental Lewy Bodies in the Absence of Clinical Parkinson's Disease

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Running head: Lewy bodies and movement abnormalities in Parkinson's disease

Key words: Lewy bodies, Parkinson's disease, movement abnormalities, aging

Supported by a grant from the United States Department of the Army (DAMD17-98-1-8621), by the National Institutes of Health (National Institute on Aging Contract N01-AG-4-2149 and grant 1 R01 AG17155-01A1, National Heart, Lung, and Blood Institute Contract N01-HC-05102, and a National Institute of Neurological Disorders and Stroke grant 1 R01 NS41265-01), and by Department of Veterans Affairs medical research funds. The information contained in this article does not necessarily reflect the position or the policy of the Government, and no official endorsement should be inferred.

Abstract

Background: Lewy body pathology is thought to predate clinical Parkinson's disease (PD). Whether common movement abnormalities associated with PD also have origins in this preclinical phase of PD development is unknown. This report examines the association between movement abnormalities in PD and incidental Lewy bodies in the absence of clinical PD.

Methods: Autopsies were performed in a sample of men enrolled in the Honolulu-Asia Aging Study following a standardized research protocol. Up to 7 years prior to death, movement abnormalities associated with PD were observed from 1991 to 1999 using the motor examination section from the Unified Parkinson's Disease Rating Scale (UPDRS).

Results: In 134 autopsied men aged 76 to 97 years at the time of death, 22 (16.4%) had Lewy bodies. Among the cases of Lewy bodies, combinations of movement abnormalities were significantly more frequent than in men without Lewy bodies. Important combinations included slow "hand movements", "rapid alternating movements of hands", "rigidity", "body bradykinesia and hypokinesia", "action or postural tremor of hands", and "tremor at rest". In the presence of 1 or fewer of these abnormalities, there were no cases of Lewy bodies (0/11). As the number of abnormalities increased, the percent of men with Lewy bodies rose significantly to 31.6% (6/19) in men with 5 or more movement abnormalities ($p=0.004$). Associations were unaltered after adjustments for age at death, past use of coffee and cigarettes, and cognitive function ($p=0.006$).

Conclusions: Findings suggest that movement abnormalities in PD are associated with incidental Lewy bodies in the absence of clinical PD. Whether combinations of movement abnormalities in individuals without PD can be used to identify subjects at high-risk for overt disease warrants further study.

Introduction

The frequency of incidental Lewy bodies (ILB), brainstem Lewy bodies in persons without clinically diagnosed Parkinson's disease (PD), ranges from five to twenty times that of overt clinical PD.(Gibb and Lees 88) Neuronal loss in the substantia nigra from brains of those with ILB has been reported to be intermediate between PD and aged normals suggesting that ILB represents a pre-clinical stage of PD in individuals who die before they develop clinical signs of PD. (Fearnley and Lees, 1991; Forno, 1969) It follows that the common extrapyramidal signs associated with PD (including tremor, rigidity, bradykinesia, and postural instability) might also present in this preclinical phase but of insufficient severity to be recognized. The autopsy component of the prospective population based Honolulu-Asia Aging Study (HAAS) provides the opportunity to examine this question. This report will investigate the association of extrapyramidal signs as measured by the Unified Parkinson's Disease Rating Scale (ref) with incidental Lewy bodies in the brains of deceased men without clinically diagnosed PD. Identification of a set of signs associated with this preclinical phase could direct approaches to screening for PD in the earliest stages when future neuroprotective interventions would be most beneficial.

Materials and Methods

Study Sample

From 1965 to 1968, the Honolulu Heart Program began following 8,006 men of Japanese ancestry living on the island of Oahu, Hawaii for development of cardiovascular disease [3,4]. At the time of study enrollment, subjects were aged 45 to 68 years. Since that time, surviving members of the original cohort have participated in repeat examinations with continuous follow-up based on a comprehensive system of surveillance that included a review of all hospital

discharges, death certificates, and autopsy records.

Beginning in 1991 focus in the Honolulu Heart Program was expanded to include research on aging and neurologic function through the establishment of the HAAS. At that time, approximately 80% (3,741) of the surviving participants in the Honolulu Heart Program received thorough physical examinations, including intentional efforts to undertake more comprehensive neurologic testing in a random subset of healthy elderly men while over-sampling high-risk subjects for dementia and cognitive impairment. Parkinson's disease and movement abnormalities were not included in the sampling strategy.

The latter effort led to standardized neurologic evaluation in 426 men, with similar procedures leading to identical evaluations in 752 participants examined from 1994 to 1996 and in 294 who were examined from 1997 to 1999. Procedures were in accordance with institutional guidelines and approved by an institutional review committee. Informed consent was obtained from the study participants. Further description of the sampling has been described elsewhere [5].

When the Honolulu-Asia Aging Study began in 1991, a specialized autopsy program was also established to describe the neuropathology of the brains in the elderly men who comprised the original cohort of the Honolulu Heart Program. While autopsies were sought on all cohort deaths, special efforts were made to perform brain autopsies in those who were demented. There were a total of 1,221 men who received the standard neurologic evaluation at 1 or more examinations. Among this group there were 134 men without PD or dementia with Lewy bodies who received an autopsy and comprise the sample of men described in this report.

Identification of Incidental Lewy bodies

Brain autopsies are performed by study neuropathologists who are shielded from clinical information according to a standardized research protocol. After fixation for 4 to 10 weeks in neutral buffered formalin, 1cm thick coronal sections of the cerebrum and 0.5 cm transverse sections of the brainstem and cerebellum are cut and examined by the neuropathologist. A standard set of tissue blocks is taken from multiple areas of the brain, including sections through the pars compacta of the substantia nigra and locus ceruleus. Hematoxilin and eosin stained sections are prepared from each of these brainstem pigmented nuclei. Both sides of one section each through the substantia nigra and locus ceruleus are examined by one of three neuropathologists for Lewy bodies. These investigators met at the onset of the study to standardize reading methods and lesion definitions and agreed to examine microscopic sections without access to clinical information.

Extrapyramidal signs and Other Confounding Information

Extrapyramidal signs associated with parkinsonsism (table 2) observed among the sampled men are based on the 14-item motor examination section of the Unified Parkinson's Disease Rating Scale (UPDRS) [6,7]. For all subjects, the UPDRS is administered by a study neurologist or a geriatrician with special training in using the UPDRS. For these analyses, an extrapyramidal sign was considered present when a subject received a score ≥1 on a UPDRS item. For those items assessed bilaterally a sign was present if either limb scored ≥ 1. It was considered absent otherwise. For men who received more than one neurologic examination, results were taken from the examination that occurred closest in time to the date of autopsy.

Other confounding information included age at death, level of education, family history of PD, cognitive function, pack-years of cigarette smoking, and coffee intake. Cognitive function

was measured at or near the time of UPDRS testing using the Cognitive Abilities Screening Instrument, developed and validated in cross-cultural studies as a comprehensive measure of intellectual capacity [8,9]. Scores range from 0 to 100 with 100 indicating optimal cognitive performance. Data on overall exposure to pack-years of cigarette smoking and to the intake of coffee were taken from examinations that occurred at the time of study enrollment (1965-1968). Coffee intake was assessed by a dietician based on 24-hour dietary recall methods [10]. Collected data were further validated against a seven-day diet record in 329 of the 8,006 men in the original cohort. Comparisons between the two measurement methods showed no significant differences in the mean intake of nine nutrients, and day-to-day variation was less than typical among western cultures [10].

Statistical Methods

For description of the observed findings, the percent of autopsied cases with Lewy bodies was estimated according to the presence and absence of an extrapyramidal sign. Tests of significance were based on exact logistic regression procedures with each extrapyramidal sign being used to predict the presence of Lewy bodies [11,12]. Estimation also provided a means for calculating the relative odds (and 95% confidence intervals) of having Lewy bodies in the presence versus the absence of an extrapyramidal sign. The percent of men with Lewy bodies was also derived according to the number of extrapyramidal signs that characterized an autopsy case among specific sets of abnormalities. In this instance, the presence of Lewy bodies was modeled through the use of logistic regression techniques with the number of movement abnormalities included as a single predictor variable. Adjustments were also made for age at death, past use of coffee and cigarettes, and cognitive function. All statistical tests were based on 2-sided levels of significance.

Results

Characteristics of the 134 autopsied men considered in this report are summarized in table 1. Among the men, 16.4% (22/134) had incidental Lewy bodies. Although differences in the characteristics were not statistically significant, cases with incidental Lewy bodies were older at the time of death than those without Lewy bodies (87.7 vs. 85.5 years, respectively). Time between the date of the examination when neurologic testing occurred and the date of death was also longer in the men with Lewy bodies versus men without Lewy bodies (3.2 vs. 2.5 years, respectively).

Pack-years of cigarette smoking and daily coffee intake at the time of study enrollment (1965-1968) tended to be less in men with Lewy bodies as compared to those without Lewy bodies. None of the men with Lewy bodies had a family history of PD while a family history was reported in 4 of the men without Lewy bodies. Level of education was similar between the two groups of men. Among the cases with incidental Lewy bodies, half (11/22) had inclusions in the substantia nigra and 86.4% (19/22) had inclusions in the locus ceruleus.

Table 2 gives the percent of men with Lewy bodies according to the presence and absence of each extrapyramidal sign from the UPDRS motor examination section. The relative odds of having Lewy bodies in the presence versus the absence of a sign is also provided. In most instances, evaluation of the 14 signs was reasonably complete. Observations were available on 11 of the 14 extrapyramidal signs in more than 90% of the autopsied men. For "postural stability", "leg agility", and "rapid alternating movements of hands", data were available on 86.6, 85.1, and 83.6% of the men, respectively. Typical reasons for incomplete testing included cognitive and physical impairment, disability, fatigue, and anxiety.

In 13 of the 14 signs examined for, the percent of men with Lewy bodies was higher in

the presence of an extrapyramidal sign versus its absence (table 2). In all but the bottom 5 extrapyramidal signs in table 2, there was at least a 2-fold excess in the odds of having Lewy bodies when an abnormality was present as compared to when it was absent.

While individually none of the relative odds were statistically significant, combinations of extrapyramidal signs were significantly more frequent in the men with Lewy bodies compared to those without Lewy bodies. The percent of men with Lewy bodies often increased significantly with an increasing number of signs. Important combinations included those that were associated with the highest relative odds of Lewy bodies that are shown in table 2. As seen in the upper left panel of figure 1, there was a higher percent of men with Lewy bodies among those with slow "hand movements" and "rapid alternating movements of hands" as compared to men with 1 or neither condition ($p=0.030$). When "rigidity" was added to this list, the association between the number of signs and the percent of men with Lewy bodies became stronger (upper right panel, $p=0.026$). Statistical significance increased further with the addition of "body bradykinesia and hypokinesia" and "action or postural tremor of hands" (lower right panel, $p=0.005$).

Among the possible sets of signs, slow "hand movements", slowing of "rapid alternating movements of hands", "rigidity", "body bradykinesia and hypokinesia", "action or postural tremor of hands", and "tremor at rest" appeared to have the strongest relation with the percent of men with Lewy bodies (see figure 2). In the presence of 1 or fewer of these signs, there were no cases of Lewy bodies (0/11). As the number of signs increased, the percent of men with Lewy bodies rose significantly to 31.6% (6/19) in men with 5 or more extrapyramidal signs ($p=0.004$). In a single individual with all 6 abnormalities, Lewy bodies were also observed. Associations were unaltered after adjustment for age at death, past use of coffee and cigarettes, and cognitive

function (p=0.006).

Other sets of extrapyramidal signs also showed significant relations with the presence of Lewy bodies, but in general, they tended to include various combinations of the 6 signs described in figure 2. "Posture", "rising from chair", "postural stability", and "facial expression" seemed to have no association with the presence of Lewy bodies in men without clinical PD.

Discussion

The prevalence of incidental Lewy bodies in the HAAS autopsy series (16.4%) is similar to other series reports when considering age. In a review pooling data from five studies the age specific prevalence of incidental Lewy bodies was 12.5%, 18.2%, and 16.7% for the seventh, eighth, and ninth decades respectively (Gibb and Lees, 1988). Morphometric studies of brains with incidental Lewy bodies have demonstrated intermediate levels of substantia nigra neuronal loss between normal aging and Parkinson's disease. Additionally, there is selectivity of neuronal loss in the lateral ventral tier of the substantia nigra identical to that of PD (Fearnley and Lees, 1991). This pathological evidence suggests that the processes leading to neuronal loss and Lewy body formation in PD and incidental Lewy body cases are similar if not identical. Whether incidental Lewy bodies represent arrested disease in affected individuals or early PD in individuals dying before onset of major motor symptoms is not known. Our results show that subjects without diagnosed PD but with extrapyramidal signs on the UPDRS are more likely to have Lewy bodies in the substantia nigra or locus ceruleus. This supports the idea that incidental Lewy bodies represent a preclinical phase of PD. Others have found mild clinical symptoms of PD in incidental Lewy body cases. For example, in an analysis of 50 cases of incidental Lewy bodies, it was found that 11 had some symptoms compatible with parkinsonism including shuffling gait or rigidity (Forno, 1969). Another report found parkinsonian features in seven of

27 cases of incidental Lewy bodies (Woodard, 1962). When we determined the per cent of individuals with incidental Lewy bodies who had extrapyramidal signs, xx% had zero, yy% had one, zz% had two etc, etc. up to six. (*Rob, if this looks good maybe we should add it to the results and add a figure)*

Our study is unique in having data on specific extrapyramidal signs collected during life in a standardized way. This allows statistical analyses that relate each of these signs singly or in combination to the odds of having incidental Lewy bodies in the locus ceruleus or substantia nigra.

These findings could have important implications for early diagnosis of PD. For example, a battery of motor tests using a combination of signs most predictive of incidental Lewy bodies could be used to screen populations and identify individuals at high risk for PD. The most predictive signs in our series were tests of coordination, motor speed, tone, and tremor. Postural instability was not predictive. This is not surprising since postural instability generally occurs late in course of PD.(Gelb, Oliver, and Gilman, 1999)

Diagnostic test batteries for PD that include motor function tasks have been developed that discriminate subjects with early PD from normal controls (Montgomery et al, 2000; Camicioli et al, 2001), and that prospectively identify subjects with suspicious parkinsonism who will develop PD (Montgomery et al, 2000B). Although these screens have not been tested in the general population, our data support this method of screening as a useful way of identifying individuals at high risk for developing PD who could participate in trials aimed at preventing or slowing the progression of PD.

Presumably, some of the autopsied men with incidental Lewy bodies could have gone on to develop clinical PD between their last Honolulu Heart Program neurological assessment and

death. This is unlikely. The Honolulu Heart Program maintains a surveillance system that tracks participants until death. A review of the hospital records on all participants whose brains were included in this study revealed none with a diagnosis of PD in the physicians' notes or problem list. The mean time between last contact with a physician and death was xx months (range yy months to zz years).

Additional concerns from the current report are associated with the general limitations that are characteristic of any autopsy study. Although sampling of the autopsied cases was more heavily weighted towards recruitment of subjects at high-risk for dementia and cognitive impairment, efforts were made to recruit neurologically healthy individuals as well. On average, the cognitive function test scores were similar in the incidental Lewy body group and the control group. Additionally, the proportion of subjects having extrapyramidal signs (based on a neurological examination administered to all subjects by a research technician) in the autopsy group versus the unselected other cohort members were similar. For example, shuffling gait was observed in 2.2% (67/3367) of non-autopsied men while it was observed in 3.8% (8/100) of those who were autopsied. Reduced arm swing was observed in 8.4% (259/3275) of the non-autopsied men while it was observed in 10.7% (16/89) in those who were autopsied. Time to walk 10 feet was slightly slower in men who received an autopsy versus those who did not (4.7 vs. 4.2 seconds, respectively).

Although there is no known cure or preventive intervention for PD, new therapies and strategies for treatment and delaying the onset of PD are likely to evolve. Early detection of PD and its preclinical stages will be critical in assessing these developments and for testing new intervention strategies. If incidental Lewy bodies truly represent preclinical PD, then extrapyramidal signs associated with their presence could broaden the population base in which

prevention and early intervention is appropriate. Approaches to prevention of PD might consider the study of subjects without PD but with features associated with incidental Lewy bodies. Whether combinations of extrapyramidal signs in individuals without PD can be used to identify subjects at high-risk for overt disease warrants further study.

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Table 1. Characteristics of the autopsied cases with and without incidental Lewy bodies.

Characteristic	Incidental Lewy bodies	
	Cases (22)*	Controls (112)
Age at death	87.7 ± 4.2† (81 - 94)‡	85.5 ± 5.5 (76 – 97)
Years to death after neurologic testing	3.2 ± 1.7	2.5 ± 1.7
Level of education¶	9.5 ± 3.7	9.3 ± 3.1
Family history of Parkinson's disease (%)	0 (0)§	3.6 (4)
Cognitive Abilities Screening Instrument	59.2 ± 17.5	58.1 ± 21.2
Pack-years of smoking at study entry	28.1 ± 28.8	32.1 ± 31.9
Coffee intake at study entry (oz./day)	10.0 ± 8.5	14.3 ± 12.1
Lewy bodies in the substantia nigra (%)	50.0 (11)	
Lewy bodies in the locus ceruleus (%)	86.4 (19)	

*Size of sample. †Mean ± standard deviation.

‡Range. §Number of cases.

¶Education is recorded as the highest level of education achieved (0 = none, 1 = primary, 2 = intermediate or junior high school, 3 = high school, 4 = technical school, and 5 = university).

Table 2. Percent of men with Lewy bodies according to the presence and absence of movement abnormalities from the motor examination section of the UPDRS.

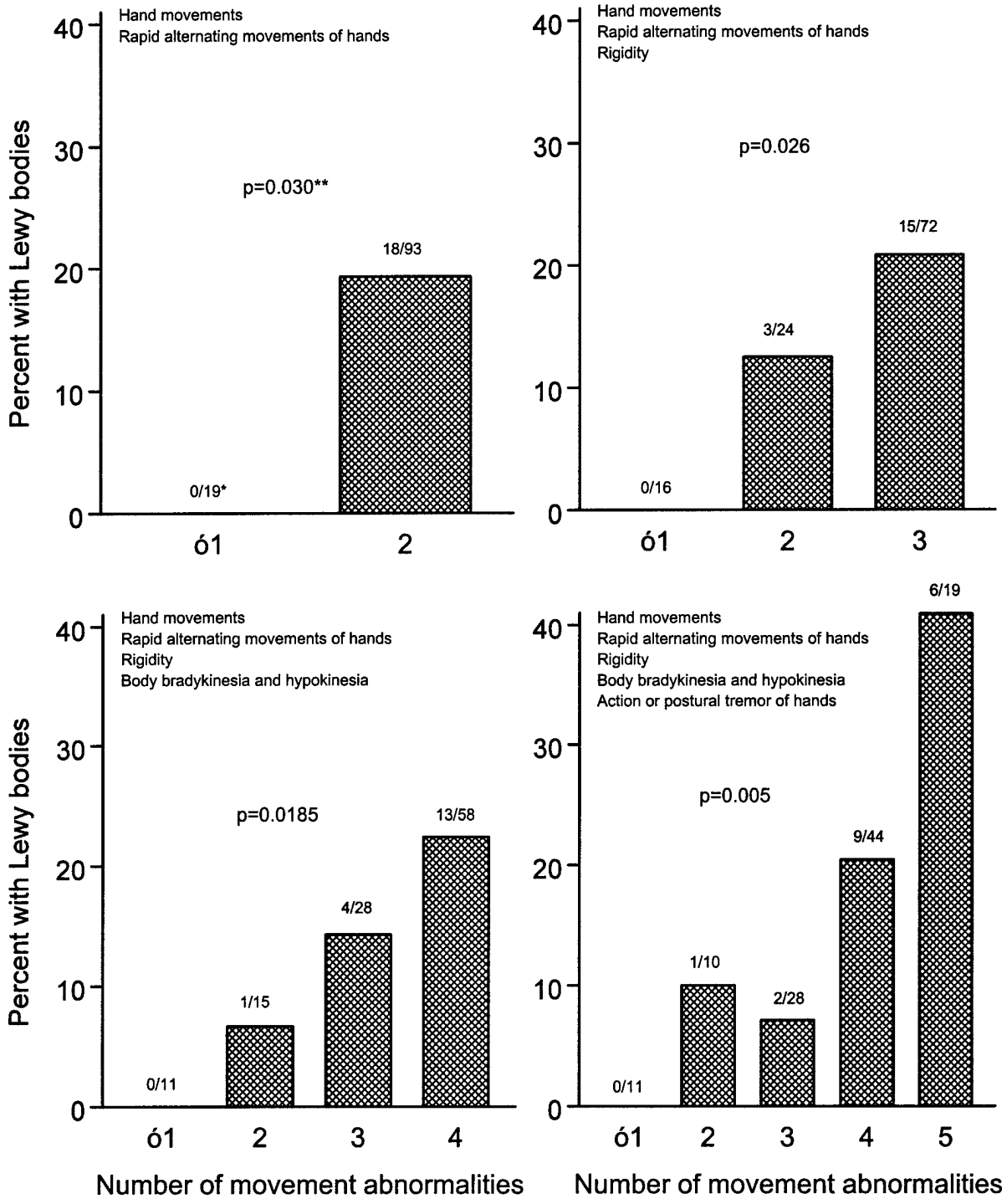
UPDRS extrapyramidal sign	Present	Absent	Relative odds
Hand movements	18.5 (20/108)*	0.0 (0/15)	4.7 (0.7,∞)†
Rapid alternating movements of hands	18.4 (18/98)	0.0 (0/14)	4.3 (0.7,∞)
Rigidity	19.6 (18/92)	7.5 (3/40)	3.0 (0.8,16.8)
Body bradykinesia and hypokinesia	19.8 (19/96)	8.1 (3/37)	2.8 (0.7,15.6)
Action or postural tremor of hands	27.3 (9/33)	12.4 (12/97)	2.6 (0.9,7.8)
Tremor at rest	33.3 (1/3)	16.0 (21/131)	2.6 (0.0,52.0)
Leg agility	19.4 (18/93)	9.5 (2/21)	2.3 (0.5,21.8)
Finger taps	18.0 (18/100)	8.7 (2/23)	2.3 (0.5,21.9)
Gait	18.8 (19/101)	10.4 (3/29)	2.0 (0.5,11.4)
Speech	18.1 (13/72)	14.5 (9/62)	1.3 (0.5,3.7)
Posture	17.3 (18/104)	16.7 (3/18)	1.0 (0.3,6.2)
Rising from chair	16.0 (12/75)	15.4 (8/52)	1.0 (0.4,3.2)
Postural stability	17.3 (13/75)	17.1 (7/41)	1.0 (0.3,3.3)
Facial expression	15.1 (11/73)	18.0 (11/61)	0.8 (0.3,2.3)

*Men with Lewy bodies/subjects at risk. †95% confidence interval.

Figure Legend

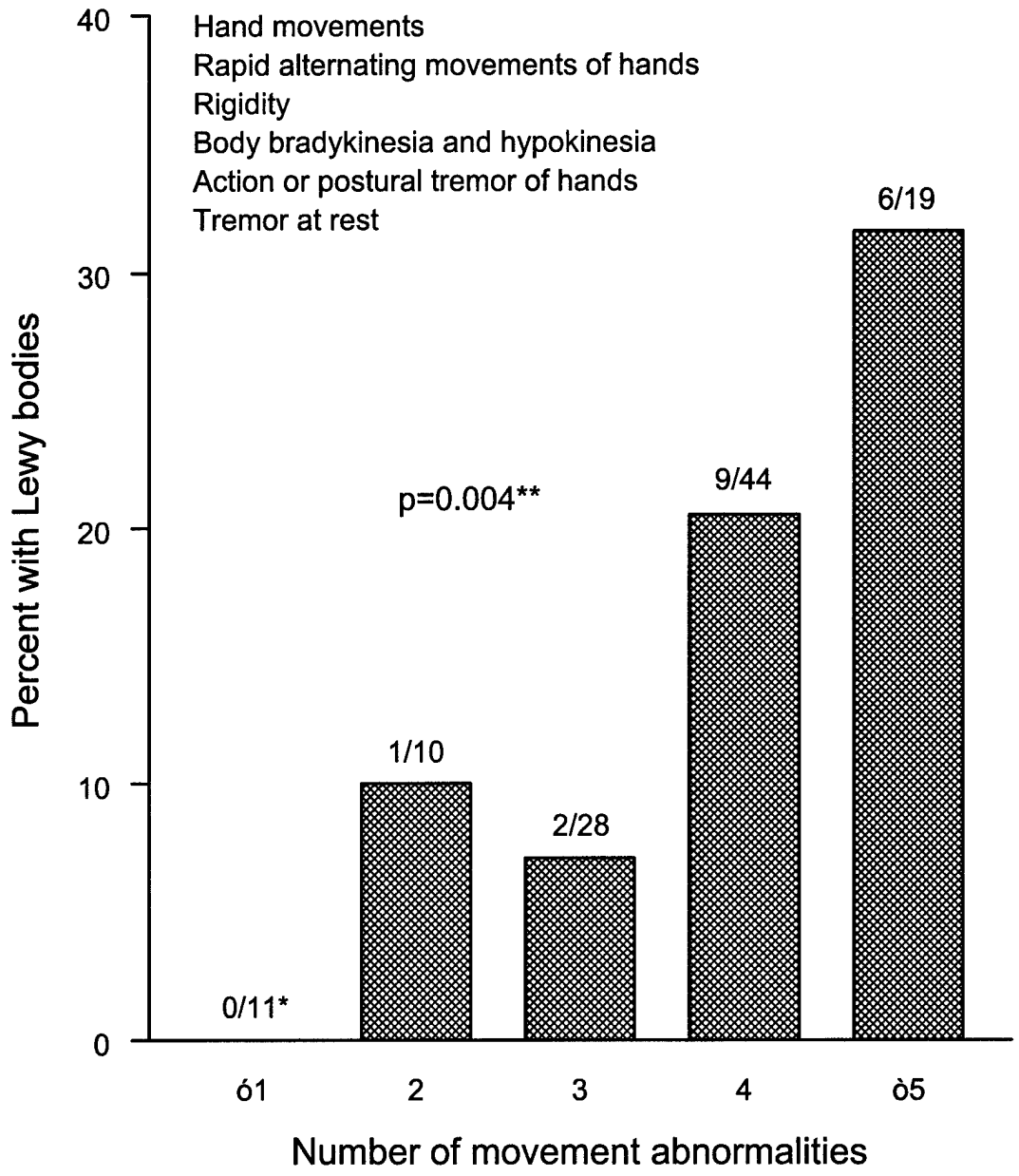
Figure 1. Percent of autopsied cases with incidental Lewy bodies according to the presence of four sets of movement abnormalities from the motor examination section of the UPDRS.

Figure 2. Percent of autopsied cases with incidental Lewy bodies according to the presence of six movement abnormalities from the motor examination section of the UPDRS.



*Subjects with Lewy bodies/sample size

**Significant increase with increasing number of movement abnormalities



*Subjects with Lewy bodies/sample size

**Significant increase with increasing number of movement abnormalities

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Journal No. Manuscript No.

APPENDIX N

Acta Neurol Scand 2002: 106: 1–6
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ACTA NEUROLOGICA
SCANDINAVICA
ISSN 0001-6314

Quantification of regional glial fibrillary acidic protein levels in Alzheimer's disease

Ross GW, O'Callaghan JP, Sharp DS, Petrovitch H, Miller DB, Abbott RD, Nelson J, Launer LJ, Foley DJ, Burchfiel CM, White LR. Quantification of regional glial fibrillary acidic protein levels in Alzheimer's disease.
Acta Neurol Scand 2002: 106: 1–6. © Blackwell Munksgaard 2002.

Objectives – Our objectives were to quantify glial fibrillary acidic protein (GFAP) in brains of Alzheimer's disease (AD) cases, and non-AD controls to determine the regions with the most severe gliosis in AD. *Material and methods* – In a case–control design, we used an enzyme-linked immunosorbent assay (ELISA) to quantify GFAP in frozen brain from four areas of neocortex in 10 AD cases, 10 age-matched controls, and 10 younger controls from the Honolulu-Asia Aging Study autopsy archive. *Results* – Median age at death was 83.5 years for cases and age-matched controls, and 77 years for younger controls. For the AD cases compared with the age-matched controls, levels of GFAP in occipital ($P = 0.01$), parietal ($P = 0.028$), and temporal lobes ($P = 0.004$) (but not frontal) were significantly higher in the cases. The median GFAP excess in AD cases compared with age matched controls was highest in the temporal lobe. *Conclusions* – Regional quantification of GFAP reveals that the glial response is most prominent in the temporal lobe in AD.

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Key words: Gliosis; Alzheimer disease; dementia; glial fibrillary acidic protein

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Accepted for publication July 3, 2002

Astrogliosis, characterized by enhanced expression of the major intermediate filament protein of astrocytes, glial fibrillary acidic protein (GFAP), is a homotypic response to brain injury. In animal models, the degree and duration of neural damage is reflected by the degree and duration of astrogliosis(1,2). Often, damage-induced elevations in GFAP as assessed by enzyme-linked immunosorbent assay (ELISA) can be observed in the absence of overt cytopathology (3). Thus, increases in GFAP serve as a sensitive and quantitative index of neural damage.

The presence of neuritic senile plaques (SP), one of the neuropathologic hallmarks of Alzheimer's disease (AD), is associated with immunohistochemical staining of GFAP in surrounding astrocytes (2). Evidence exists that the colocalization of astrocytes with SP in the hippocampus occurs early in AD, often in the absence of dystrophic neurites suggesting that astrogliosis may not just be a response to neuronal injury but may also contribute to the AD process (3). In the neocortex, levels of GFAP messenger RNA (mRNA) have been reported to be correlated with SP density in temporal but not frontal neocortex in AD brains (4). Complicating these observations, animal research has shown that levels of GFAP and GFAP mRNA in brain tissue increase with age (5). Thus, it is necessary to consider the effects of aging when evaluating the association between GFAP levels in the brain and presence of AD.

Using a sandwich ELISA, we quantified GFAP levels without knowledge of diagnosis in four areas of neocortex in brains from individuals with AD, age-matched controls, and younger controls with normal cognition prior to death. We speculated that not only would levels of GFAP be higher in the brains of AD subjects, but that the GFAP elevations would be highest in the neocortical region thought to be involved earliest in the AD process – the temporal lobe (6).

Material and methods

The study population

The Honolulu-Asia Aging Study (HAAS) began in 1991 as a supplement to the Honolulu Heart Program, a longitudinal study of cardiovascular disease in a cohort of Japanese-American men living on Oahu at the time of the baseline examination in 1965. The original cohort consisted of 8006 men born 1900 through 1919. Detailed descriptions of the study design have been previously published (7, 8).

Dementia case-finding methods

Evaluation and follow-up for dementia began at the 1991–1993 examination of the cohort when the men were 71–93 years (average 78 years) of age and a second round of evaluations was carried out from 1994 to 1996. All participants received the Cognitive Abilities Screening Instrument (CASI) that has been validated and used in USA and Japan for evaluating cognitive function (9, 10). Scores ranged from 0 to 100 with 74 identified as the optimal score distinguishing demented from non-demented (11). Participants signed informed consent forms at each examination. The multi-step procedure used to identify cases of dementia has been described in detail elsewhere (9). Subjects suspected to have dementia based on poor performance on the CASI received a full diagnostic evaluation for dementia. This included a standardized interview and a neurological examination by a neurologist, as well as the neuropsychological test battery from the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) (12, 13). Those individuals judged by the study neurologist to meet *Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised* (*DSM-III-R*) (14) criteria for dementia had brain computed tomographic scans and blood tests including complete blood count, chemistry profile, vitamin B12 level, red blood cell (RBC) folate level, rapid plasma reagin, and thyroid function test.

Final diagnosis and Clinical Dementia Rating index (15) were assigned by a clinical consensus committee that included the study neurologist and at least two other investigators.

Diagnosis of AD was made using the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria (16).

Autopsy methods

A concentrated effort to obtain autopsies on all cohort deaths also began in 1991. The idea of autopsy was discussed with each participant. In the state of Hawaii the family must give final consent for autopsy after death. Research protocol autopsies are currently obtained on greater than 20% of all cohort deaths.

Brains were removed by a neuropathologist or neuropathology technician within 30 h of discovery of death (average time from death to autopsy was 14.6 h). Immediately following removal of the brain, tissue was resected (usually from the left hemisphere) for storage at −70°. For this study, frozen tissue from four brain regions: the frontal pole, temporal pole, superior lateral parietal cortex 2 cm posterior to the motor cortex, and the occipital pole were used. Gross examination of the brains was carried out by a neuropathologist (JH) who was blinded to the participant's clinical history. The microscopic examination included close inspection of sections of frontal, temporal, parietal, and occipital cortices, as well as CA1 and subiculum of the hippocampus stained with H&E and Bielschowsky stains and stains using antibodies directed against β-amyloid and ubiquitin. Microscopic sections were examined by one of the three study neuropathologists without knowledge of the clinical findings. Lesions including SP, neuritic plaques, and neurofibrillary tangles were counted per mm^2 (17) in five fields selected as having the most lesions for each of the four neocortical areas. The highest count among the five fields was taken to represent that area.

Study design

For this case–control study, 10 AD cases meeting NINCDS-ADRDA clinical criteria for definite AD and meeting CERAD neuropathological criteria for definite or probable AD were selected from the HAAS autopsy archive. Ten age matched men without dementia were selected who scored 74 or higher on the CASI within 5 years of death (mean interval between CASI and death was 25 months) and who had less than two neuritic plaques per mm^2 in the neocortex at autopsy. A third group of 10 younger controls were selected based on age less than 80 years at death, cognitive function score 74 or higher and having zero neuritic plaques in the neocortex at autopsy.

GFAP assay and immunoblots

Frozen tissue blocks were removed from the storage tubes and held frozen on a cold plate.

With the aid of a no. 11 scalpel, 25–50 mg of gray matter from each neocortical block were excised, weighed, and homogenized by sonification in 10 volumes of hot (90–95°C) 1% (w/v) sodium dodecyl sulfate (SDS).

Assays were performed without knowledge of case status or neuropathologic findings. Aliquots of the homogenates were assayed for GFAP using the sandwich ELISA of O'Callaghan (18). Briefly, a rabbit polyclonal antibody to GFAP (Dako Corp., Carpenteria, CA, USA) was coated on the wells of Immulon-2 microtiter plates (Dynatech Laboratories, Chantilly, VA, USA). After blocking non-specific binding with non-fat dry milk, aliquots of the homogenates were diluted in sample buffer and added to the wells of the plate. After appropriate blocking and washing steps, a mouse monoclonal antibody to GFAP (Chemicon, Temecula, CA, USA) was added to "sandwich" GFAP between the two antibodies. An alkaline phosphatase-linked antibody directed against mouse immunoglobulin G (IgG) (Dako Corp., Carpenteria, CA, USA) was then added, and a colored reaction product was obtained by subsequent addition of enzyme substrate. Quantification was achieved by spectrometry at 405 nm using a microplate reader (UV Max running on a Soft Max program, Molecular Devices, Menlo Park, CA, USA). Data are expressed as μg GFAP per mg total protein. This assay of GFAP has been cross-validated with another solid-phase immunoassay (18) and with densitometric analysis of Coomassie blue-stained GFAP resolved by two-dimensional electrophoresis (19).

GFAP immunoblots were performed to assess GFAP integrity in the same samples subjected to ELISA. Aliquots of the sample homogenates were resolved by SDS–polyacrylamide gel electrophoresis (PAGE), transferred to nitrocellulose membranes (Schleicher & Schuell, Keene, NH, USA) and subjected to immunoblot analysis according to the procedure described by O'Callaghan (20). Detection of the immunoreactive bands was achieved using the [^{125}I] rProtein-A and the same monoclonal anti-GFAP antibody used in the GFAP ELISA.

Statistical methods

Because of the small sample size within each of the three groups, non-parametric methods were used to describe and analyze the data. Comparisons between cases and age-matched controls for each region of the brain were made using the difference in GFAP levels between a case and an age-matched control based on the Wilcoxon signed rank test. These comparisons form an attempt to assess differences in GFAP levels within a region of the brain that can be attributed to AD and not to age. Within-region differences between AD cases and younger controls were also made, but here, significance testing relied on a Wilcoxon rank sum test (21).

Results

Distributional characteristics of selection variables (last CASI score, maximum neurofibrillary tangle count, maximum neuritic plaque count) and the matching variable (age) confirm expected differences and similarities among the three groups (Table 1). The distribution of years of school

Table 1. Median levels of selected characteristics for the control groups and for the cases of Alzheimer's disease

Characteristic	Controls		Cases†
	Young*	Age-matched	
Age (years)	77.0 (76–79)‡	83.5 (76–94)	83.5 (76–92)
Education (years)	12 (8–16)	9.5 (6–20)	8 (7–12)
Most recent CASI score	85.3 (82.9–93)	86.3 (73.5–93)	4.7 (0–75.2)
Post-mortem interval (h)	14.5 (4.1–23)	13.3 (4.9–24.1)	9.2 (6.1–30.6)
Maximum NFT count	1.1 (0–2.7)	0.5 (0–13.5)	44.4 (0–99)
Maximum NP count	0 (0–0)	0 (0–1.5)	9.7 (5.2–17)
GFAP (μg/mg total protein)			
Frontal	10.0 (4.2–22.6)	12.7 (1.5–27.6)	10.5 (7.8–44.1)
Occipital	5.8 (1.5–11.1)	3.6 (0.7–9.4)	11.9 (3.5–26.0)
Parietal	8.3 (0.3–17.5)	8.9 (3.3–23.3)	14.7(7.2–47.5)
Temporal	12.3 (4.0–22.8)	11.1 (2.9–41.4)	39.7 (8.1–77.5)

* Other than age, there were no significant differences between the two control groups for any of the other characteristics.

† Other than the selection criteria used to identify cases of Alzheimer's disease (most recent CASI score and maximum number of NP and NFT), there were no significant differences in any of the other characteristics between the cases of Alzheimer's disease and their age-matched controls except for levels of GFAP that were observed in the occipital, parietal, and temporal lobes ($P = 0.010$, $P = 0.028$ and $P = 0.004$, respectively).

‡ Range.

attendance is highest among younger controls and lowest among cases of AD ($P = 0.08$). Although the median value of the post-mortem interval is notably smaller among cases of AD, the overall distributions of the interval among the three groups are indistinguishable ($P = 0.61$). There is no statistically significant association between post-mortem interval and GFAP levels in the control subjects (the lowest P-value for this relationship was in the temporal lobe; $P = 0.17$).

Frontal lobe GFAP levels are indistinguishable among the three groups (Fig. 1). However, for the remaining three regions (occipital, parietal, temporal), the GFAP levels are significantly higher for cases of AD vs their age-matched controls (Tables 1, $P = 0.010$, 0.028, 0.004, respectively). Furthermore, 80–90% of cases of AD have higher GFAP levels compared with their age-matched controls in occipital, parietal, and temporal regions (Fig. 2). Levels of GFAP are indistinguishable between the two control groups for all three of these brain regions. The highest measurements of GFAP were observed in the temporal region among cases of AD and the greatest GFAP excess in cases of AD vs age-matched controls was in the temporal lobe (Fig. 2). Results are essentially unchanged after removing from the analysis those subjects with a post-mortem interval greater than 18 h.

The GFAP immunoblot results provide additional confirmation for the ELISA method. A consistent band pattern was observed for all samples. A major band appears at approximately 50 kDa, consistent with the known molecular

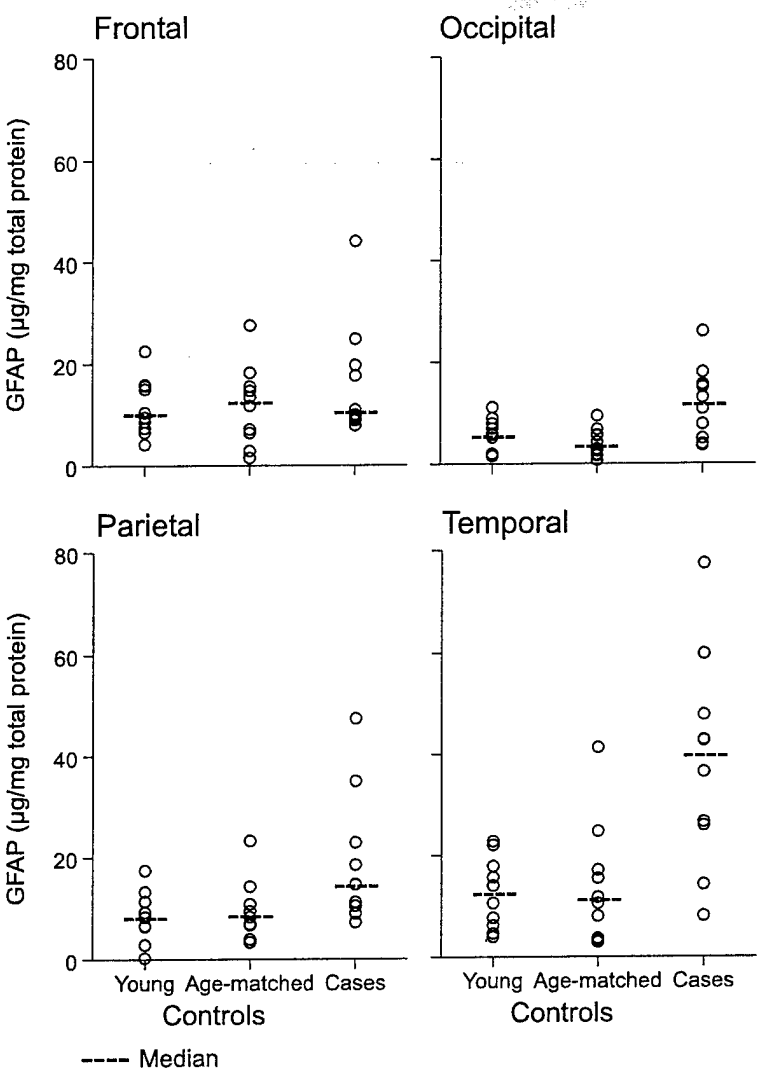

Figure 1. Individual GFAP levels in the two control groups (young and age-matched controls) and in the cases of Alzheimer's disease within each region of the brain.

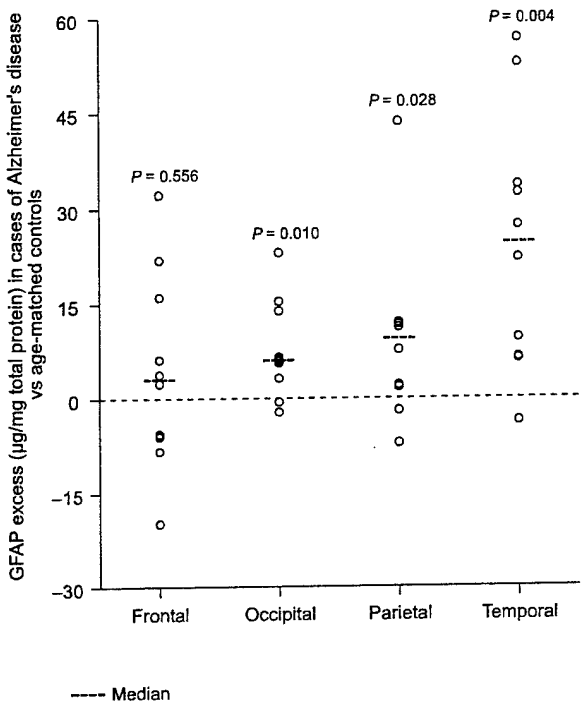

Figure 2. Excess individual GFAP levels in the cases of Alzheimer's disease as compared with their individually age-matched controls within each region of the brain. Reported *P*-values correspond to statistically significant excesses of GFAP levels in the cases of Alzheimer's disease.

weight of GFAP (data not shown). Three to four minor immunoreactive bands are resolved below the major band corresponding to GFAP turnover products seen in experimental animals. For one of the 30 subjects, GFAP ELISA data for all brain regions are very low; immunoblots of these same samples reveal very low immunoreactivity and a loss of the 50 kDa band – findings indicative of proteolysis (post-mortem interval was 24.1 h). Exclusion of this individual from statistical analyses had no effect on findings.

Discussion

Astrogliosis is a well known neuropathologic feature in AD and there is evidence supporting the association of reactive astrocytes with SP (22, 23). The temporal sequence of plaque formation, amyloid deposition, neuronal loss, and astrogliosis is not known; however, there is evidence that astrogliosis occurs early in AD perhaps in response to fibrillar Abeta deposits. This suggests that astrogliosis may contribute to the AD process (3).

To our knowledge, this is the only report of quantitative differences in GFAP levels across regions of the human brain that are associated with AD. Using an immunoassay procedure to quantify levels of GFAP, we confirmed that GFAP is elevated in the brains from subjects with AD and demonstrated that the greatest elevations are in the temporal lobe. The more than threefold increase in temporal lobe GFAP found in this analysis of AD cases corresponds to a large degree of neural damage in this region.

While median GFAP levels were significantly higher in temporal, parietal, and occipital cortices of the AD brains compared with both control groups, no significant differences were found for the frontal cortex. These findings support another report (4) where GFAP mRNA levels were elevated in the temporal cortex of elderly women with AD and correlated positively with SP density while levels in the frontal lobe were not associated with SP density. It is notable that the regional pattern of elevated GFAP in the AD brains roughly corresponds to the marked hypometabolism of glucose predominantly involving the temporal and parietal lobes seen on positron emission tomography (PET) in patients with AD (24, 25). A similar pattern of neocortical hypometabolism has been seen in PET imaging of the non-human primate in response to a neurotoxic injury to the entorhinal cortex (26). In this study, there was a significant association between the degree of damage in the entorhinal cortex and the deficits in metabolism.

We observed no differences in the GFAP levels between the age-matched and young controls. This finding is counter to most reports in humans that show increased GFAP mRNA in the hippocampus, frontal and temporal cortex after the age of 60 in unaffected brains (27), and increases in GFAP in cerebrospinal fluid (CSF) with increasing age (28). Failure to find any difference may be because of limited age range and small sample size. Age related GFAP elevations might have already developed in our controls, the youngest of whom was 76 years.

Astrocytes are activated by proinflammatory mediators (cytokines, chemokines) expressed by activated microglia and macrophages. Neurotrophic factors [transforming growth factor-β (TGF-β)] and prostaglandins known to activate astrocytes are also elaborated by activated microglia (29). While astrocyte activation and subsequent gliosis has a putative role in healing following injury to the central nervous system, glial scarring may also have a detrimental effect on neuronal function (30). This hypothesis may be especially relevant to the pathogenesis of AD. Colocalization of SP with reactive astrocytes but not dystrophic neurites supports the idea that astrogliosis may occur prior to and contribute to

neuronal injury in AD (3). Inhibition of GFAP synthesis may delay astrogliosis and allow for axonal regeneration and remyelination. In the future, manipulation of factors that activate astrocytes may be an additional strategy for treating or preventing AD.

Acknowledgements

This work was supported in part by National Institute on Aging contract NO1-AG-4–2149; the United States Department of the Army grant DAMD17-98-1-8621, the Office of Research and Development Medical Research Service, Department of Veterans Affairs, and a contract from the National Institute of Occupational Safety and Health.
The information contained in this article does not necessarily reflect the position or the policy of the Government, and no official endorsement should be inferred.

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Associations of Cortical Astrogliosis with Cognitive Performance and Dementia Status: The Honolulu-Asia Aging Study

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Number of Pages: 22
Number of References: 44
Number of Figures: 1
Number of Tables: 2

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Running Head: Astrogliosis and Cognitive Performance

Abstract

Immunohistological evidence has linked the presence of glial fibrillary acidic protein (GFAP), a marker for astrogliosis, to regions of the brain possessing the pathological features associated with Alzheimer's disease. However, astrogliosis is 1) not limited to these regions, 2) occurs in response to a variety of damaging stimuli to the brain, and 3) is not specific to the agent causing the damage. Given these observations and the non-quantitative nature of immunohistochemistry, the present investigation utilized a sensitive ELISA technique to quantify GFAP within four specific cortical brain regions and to assess possible associations between GFAP and 1) a measure of cognitive function, 2) several clinical dementia conditions, and 3) neuritic plaque (NP) and neurofibrillary tangle (NFT) neuropathology. We examined 204 decedents of the autopsy component of the Honolulu-Asia Aging Study, a longitudinal cohort study, who had been clinically assessed for dementia several times prior to death. Our results indicate that cognitive function is inversely associated with GFAP in the occipital, parietal and temporal lobes, but not in the frontal lobe. This relationship remains significant when adjusted for NP and NFT counts. Further, compared to a non-demented group of individuals, significantly greater GFAP levels are found in those diagnosed with Alzheimer's disease, vascular mediated dementia, and those identified as having mixed dementia. These findings underscore the need to look beyond standard neuropathological measures putatively linked to specific neuropathological conditions in efforts to identify common cellular and molecular processes that contribute to dementia.

Keywords: astrogliosis, cognitive performance, dementia, Alzheimer's disease

1. Introduction

Of the population over age 65, approximately 8-10% suffer from dementia [1,2]. As a public health problem of major proportion, the continuing increase in the number of elderly will only add to the significance of the problem. There are many types of dementia, each with relatively unique clinical features and neuropathological characteristics [3]. A number of pathological processes may co-exist and contribute additively, if not synergistically, to cognitive decline. Indeed, the processes related to vascular pathology as well as those underlying Alzheimer's disease often appear together, and dementia is often observed in the less common neurodegenerative conditions such as Parkinson's Disease [4,5].

The etiology is unknown for the most common cause of dementia, Alzheimer's disease. Neuropathological confirmation of Alzheimer's disease depends on the presence of amyloid containing NP's in neocortical regions, and to a lesser extent, on the presence of NFT's. However, post-mortem studies indicate that NP's and NFT's may be present in the brains of clinically normal individuals [6-9]; and that patients clinically labelled with Alzheimer's disease may not have an Alzheimer-like neuropathology [10]. This suggests that there are other pathological processes occurring in the brains of these individuals which contribute to their overt dementia.

Neuropathological alterations are taken as evidence of an adverse effect and such changes are presumed to serve as the underlying basis for dementia. However, in the absence of specific hallmarks of a dementia-associated disease (such as the NP's and NFT's associated with Alzheimer's disease), subjective identification of sparse, highly dispersed, or novel neuropathological changes is not always possible. For example, selective damage to a specific population of axons or their terminals would escape detection with traditional histological stains

(e.g., H & E, Nissl) that predominantly reveal neural perikarya; yet such undetected damage could underlie the demented condition.

One approach to overcome the problem of "missed" sites of damage is to monitor astrogliosis. Astrogliosis (reactive gliosis) is the non-specific response of astrocytes to all types of brain injuries and disease states. It is characterized by the accumulation of glial filaments, a major constituent of which is the intermediate filament protein, glial fibrillary acidic protein (GFAP). Thus, quantitative assessment of GFAP as an index of astrogliosis provides an indirect means to detect and quantify sites of brain damage. Indeed, animal studies have demonstrated that the onset, degree, duration, and regional localization of GFAP directly reflects the onset, degree, duration, and region of damage to the nervous system following a variety of toxic insults [11,12]. Increases in GFAP have proven to be sensitive and early indicators of the sites of neural damage, even in the absence of overt histopathology. Damage to axons [13], cell bodies [14], and nerve terminals [15,16] all result in the induction of GFAP. Thus, induction of GFAP may constitute an underlying process common to a variety of neurodegenerative diseases that serves as a pathophysiological marker regardless of the underlying molecular or cellular basis for the disease condition.

While astrogliosis has been widely documented in postmortem tissue from victims of Alzheimer's disease, relatively little evidence has been obtained for reactive gliosis associated with dementia and cognitive decline in the absence of AD. Furthermore, little attention has been directed toward quantification of GFAP; most studies have been limited to evaluations of astrogliosis by GFAP immunohistochemistry. Attempts to quantify astrogliosis by employing cell counts are flawed because astrogliosis is characterized by accumulation of more GFAP per astrocyte and not the accumulation of more astrocytes [14,17-21]. Ultimately, small but

pathologically relevant increases in GFAP have likely been missed in most morphology-based studies; primarily because the method is comparatively insensitive.

To address these limitations the present investigation focussed on the quantification of astrogliosis in brains of decedents from the autopsy component of the Honolulu-Asia Aging Study who had been clinically assessed for dementia several times prior to death. The objective of this investigation was to assess the relationship between GFAP and cognitive performance, several clinical dementia conditions, and NP and NFT neuropathology from 4 cortical brain regions. This study also allows the association between standard neuropathology measures and levels of GFAP to be evaluated using a more precise and sensitive estimate of astrogliosis.

2. Materials and Methods

Study Population

The Honolulu-Asia Aging Study (HAAS) began in 1991 as a supplement to the Honolulu Heart Program. The original program is a longitudinal study of cardiovascular disease in a cohort of Japanese-American men living on Oahu at the time of the baseline examination in 1965. The cohort consisted of 8006 men born between 1900 and 1919. Detailed descriptions of the study design have been previously published [22-25].

Dementia case-finding methods

Evaluation and follow-up for clinical dementia began during the fourth examination (1991 to 1993) of the cohort when the men were 71 to 93 (average 78) years of age and a second round of evaluations was conducted from 1994 to 1996. At each examination participants signed an informed consent document. Most participants received the Cognitive Abilities Screening Instrument (CASI) that has been validated and used in United States and Japan to evaluate

cognitive function [25,26]. Scores ranged from 0-100 with 74 identified as the optimal score distinguishing demented from non-demented individuals [27]. A multi-step procedure was used to identify cases of dementia, and is described in detail elsewhere [25]. Based on poor performance on the CASI, subjects suspected to have dementia received a full diagnostic evaluation. This included a standardized interview, an examination by a neurologist, and the neuropsychological test battery from the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) [28,29]. Final diagnosis and Clinical Dementia Rating Score [30] were assigned by a clinical consensus committee that included the study neurologist and at least two other investigators.

Six mutually exclusive categories of participants were identified.

1) Based on criteria characterizing clinical dementia in the previously published report on this cohort [25], a category was created for which Alzheimer's disease (AD) is likely (Likely AD) based on the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria [31]. Pathologic findings were not taken into account for these criteria.

2) A category was created for which vascular dementia (VsD) is likely (Likely VsD) based on clinical criteria proposed by the California Alzheimer's Disease Diagnostic and Treatment Centers (ADDTC) [32].

3) Categorization of mixed dementia (Likely MD) included several types. Mixed dementia with likely AD was defined as possible AD with a second clinical disorder sufficient to cause dementia. Mixed dementia with a high likelihood of a vascular component was defined according to ADDTC criteria. Other mixed cases were defined using the judgement of the consensus panel.

4) Mild cognitive impairment (Mild CI) was defined as those remaining individuals with a score on the last CASI of less than 82.

5) A category defined as No Dementia included those remaining men who did not meet any clinical criteria for categorization into one of the three aforementioned dementia categories *and* who scored 82 or higher on the last CASI.

6) An additional unclassified category (Unclassified) included cohort men who did not participate in any clinical evaluation but who came to autopsy upon their death.

Clinical categorization did not involve data derived from neuropathology assessment. Thus, the three clinical dementia categories (Likely AD, Likely VsD, Likely MD) derived from the consensus panel should be viewed as a grouping process identifying a high likelihood of definite AD, of definite VsD, or of definite mixed dementia. However, it should be recognized that some degree of misclassification is inherent in this method of diagnosis [33].

Autopsy Methods

A concerted effort to obtain autopsies on all cohort deaths also began in 1991. Research protocol autopsies are currently obtained on greater than 20% of all cohort deaths. A total of 327 decedents underwent autopsy from 1992 to 1999. Of these, 232 had measures of GFAP obtained and 204 of these individuals had a complete neuropathology assessment. Only those 204 where measures of GFAP were obtained and who had the complete neuropathological assessment were utilized in the analyses.

Brains were removed by a neuropathologist or neuropathology technician within 30 hours of discovery of death (average time from death to autopsy was 14.6 hours). Immediately following removal of the brain, tissue was dissected from different areas (usually from the left hemisphere) for storage at -70° C. For this study, frozen tissue (0.4-2.0 g) was used from each of four brain regions: the frontal pole, temporal pole, superior lateral parietal cortex two centimeters posterior to the motor cortex, and the occipital pole. Gross examination of the brains was done by a

neuropathologist who was blinded to the participant's clinical history. The microscopic examination included close inspection of sections of the middle frontal gyrus, superior middle temporal gyri, inferior parietal lobule, and occipital association cortex along the calcarine sulcus stained with H&E and Bielschowsky stains, and stains using antibodies directed against □-amyloid and ubiquitin. Microscopic sections were examined by one of three study neuropathologists without knowledge of the clinical findings. Lesions including senile plaques, NP's, and NFT's were counted per mm^2 [34] in 5 fields selected by the neuropathologist as having the most lesions for each of the 4 neocortical areas.

GFAP Assay

Frozen tissue blocks were removed from the storage tubes and held frozen on dry ice until homogenization. With the aid of a # 11 scalpel, 25-50 mg of gray matter from each neocortical block was excised, weighed, and homogenized by sonication in 10 vol of hot (90-95 °C) 1% (w/v) sodium dodecyl sulfate (SDS).

Assays were performed without knowledge of case status or neuropathological findings. Aliquots of the homogenates were assayed for GFAP using a sandwich ELISA [35,36]. Briefly, a rabbit polyclonal antibody to GFAP (Dako Corp., Carpenteria, CA) was coated on the wells of Immulon-2 microtiter plates (Dynatech Laboratories, Chantilly, VA). After blocking nonspecific binding with non-fat dry milk, aliquots of the homogenates were diluted in sample buffer and added to the wells of the plate. After appropriate blocking and washing steps, a mouse monoclonal antibody to GFAP (Chemicon International, Temecula, CA) was added to "sandwich" GFAP between the two antibodies. An alkaline phosphatase-linked antibody directed against mouse IgG (Dako Corp., Carpenteria, CA) was then added, and a colored reaction product was obtained by subsequent addition of enzyme substrate. Quantification was achieved by

spectrometry at 405 nm using a microplate reader (UV Max running on a Soft Max program, Molecular Devices, Menlo Park, CA). Data are expressed as □g GFAP per mg total protein. This assay of GFAP has been cross-validated with another solid-phase immunoassay [37] and with densitometric analysis of Coomassie blue-stained GFAP resolved by two-dimensional electrophoresis [14].

Statistical analysis

Statistical analyses examined how measures of cognitive function, clinical dementia classification and neuropathological variables are related to levels of GFAP within specific brain regions. To this end and for all analyses, GFAP is statistically utilized as the dependent variable, and cognitive, clinical and neuropathological variables are utilized as independent variables. This approach allows us to determine how standard measures of dementia might be associated with differences in the level of GFAP when analyzed as single variables, as well as jointly using cognitive performance, clinical diagnosis and neuropathological data. This approach in no manner assesses causality or temporal sequence; these issues being a matter of study design and not statistics. All statistical analyses were performed using the SAS system (SAS Institute, Cary, NC). GFAP levels were transformed using the natural logarithm [ln(GFAP)] to produce a normally distributed random variable. These values were converted back by exponentiation to original units for the figure and tables. The maximum of the 5 counted fields for NP and NFT counts within a brain region was used for statistical analyses of the neuropathology variables.

Linear regression analysis was utilized to model the relationship between GFAP and CASI scores, NP and NFT counts. One-way ANOVA's were utilized for analyses involving the categorical clinical diagnoses. Pairwise comparisons between the No Dementia group and all other classifications were performed using Dunnett's Test.

Single variable models were assessed to estimate regression coefficients for CASI score, clinical diagnosis, NP's, NFT's, and age. Multivariable analyses were performed to assess the relationship between GFAP and measures of cognitive function or clinical diagnosis while adjusting for differences in levels of NP's and NFT's. These analyses provide an estimate of the strength of association between GFAP and cognitive function in a situation where co-relations with NP's and NFT's have been removed. A model containing only neuropathology measures (NP and NFT counts) served as the initial model (Model 1). Model 2 was formed by adding to Model 1 the assessment of cognitive function in the form of the CASI score. Significant coefficients for CASI indicate that this measure of cognitive function is able to explain differences in GFAP levels beyond those accounted for by differences in NP and NFT counts. Model 3 was formed by adding clinical information in the form of the clinical diagnosis to Model 1.

Two additional models were examined to determine if after the inclusion of neuropathology and clinical variables there was residual variation in GFAP which could be accounted for by age. The variable age was added to Model 2 and Model 3, and tested for statistical significance.

3. Results

Descriptive/Univariate Analyses

Mean age, CASI scores, and levels of GFAP for each clinical classification are presented in Table 1 . Compared to the No Dementia group, those in the clinically demented categories, including those with Mild CI, are significantly older, and have significantly lower CASI scores. Statistically significant elevations in GFAP levels were observed in all brain regions except the frontal lobe; the largest elevations were observed in the Likely AD category. However, the Likely MD and the Likely VsD groups also were significantly elevated above their corresponding No

Dementia groups in some brain regions. The largest GFAP values were seen in the temporal lobe where two-fold greater increases were observed for the Likely AD category compared to the No Dementia group.

Univariate scatterplots showing the relationship between CASI score and levels of GFAP in each brain region are shown in Figure 1. The concentration of GFAP varied inversely with CASI scores in all four brain regions examined. This relationship was statistically significant in all four brain regions (Table 2).

Statistically significant relationships between GFAP levels and both NP and NFT counts existed in all cortical regions with the exception of the frontal lobe (Table 2). Regression coefficients for all of these relationships are positive, and the value of R^2 is greatest in the temporal lobe.

Multivariable Analyses

Three statistical models were used to assess the impact of NP and NFT counts, CASI scores, and clinical dementia categories on differences in levels of GFAP (Table 2). The initial joint model examining the relationship between the NP and NFT measures and GFAP (Model 1) was statistically significant in all brain regions with the exception of the frontal lobe.

Model 2, which contained the addition of the CASI score to a model containing NP and NFT measures, was statistically significant in all 4 brain regions. The ability of the CASI score to account for variation in GFAP levels is further reflected in 1) the significant regression coefficients for the CASI score in each region, 2) a decrease in the magnitude of the coefficients for NP's and NFT's, and 3) the increase in the value of the R^2 relative to Model 1.

When clinical diagnostic categories are added to the model containing neuropathology measures (Model 3), the overall model is statistically significant in all regions except for the frontal

lobe. This model demonstrates the relationship between GFAP and clinical dementia categories after adjusting to a common NP and NFT density count for all six clinical categories.

Only in the temporal lobe does the addition of clinical information to the model containing neuropathology significantly improve the fit of the model. Adjusting for NP's and NFT's, there is a 26% reduction in the difference between Likely AD and No Dementia (12.56 to 9.34 □g GFAP/mg total protein), but only an 8.5% reduction for Likely MD vs No Dementia (7.12 to 6.49 □g GFAP/mg total protein). However, the adjusted differences in mean GFAP levels remain statistically significant. Similar reductions are seen in the parietal lobe and the occipital lobe, however these differences are not statistically significant.

Compared to values unadjusted for NP and NFT counts, the differences in GFAP between Likely VsD and No Dementia remains remarkably constant (<10% attenuation) in all brain regions, statistically significant in the occipital lobe, and notable in the parietal lobe (p=.0832) when adjusted for NP and NFT counts (Table 2). Indeed, even in the frontal lobe, wherein no GFAP difference among groups appears to be particularly notable, the largest difference is observed between Likely VsD and No Dementia (p=0.0512 unadjusted for NP and NFT counts, and p=.070 adjusted).

Only in the occipital lobe was age able to significantly account for any additional variability in GFAP. Regression coefficients for age were 0.0187 (p=.007), and 0.0202 (p=.0057) for models containing neuropathology and the CASI score or the clinical classification respectively.

4. Discussion

We have shown that cortical levels of GFAP, a direct marker of astrogliosis, correlates inversely with measures of cognitive performance, and is associated with categories of clinically

determined dementia. Indeed, increases in astrogliosis are not limited to those thought to be afflicted with AD, but also include those with mixed dementia and those with vascular mediated dementia. Enhanced expression of GFAP, as an index of astrogliosis, is known to reflect underlying neuropathology in the absence of overt cell loss or damage. As such, our data suggest that the clinically demented condition is associated with underlying neuropathology that remains to be defined at the molecular and cellular level. Further, our data also demonstrate that evaluation of GFAP levels in very small samples of human brain provides a simple means of quantifying the degree and location of covert neuropathology that correlates to deficits in brain function.

Previous studies have shown associations between standardized density counts of NP's and NFT's, and both qualitative and quantitative measures of astrogliosis [38-41]. Our analyses confirm these relationships. Above-and-beyond this association with NP and NFT measures, the study uniquely identifies clinical dementia information as a significant correlate of astrogliosis. This quantification of astrogliosis through ELISA measurement of GFAP suggests the potential role of astrogliosis as an important common element of pathological processes among a variety of causes of neural insult and disease leading to dementia. In aggregate, findings suggest that NP and NFT pathology is most notably associated with direct quantitative markers of astrogliosis 1) in the two brain regions (temporal and parietal) typically associated with the pathology of confirmed Alzheimer's disease, *and* 2) in the two clinical dementia categories (Likely AD, Likely MD) wherein the likelihood of definite Alzheimer's disease is highest. Residual elevations in GFAP in the temporal lobe, independent of NP and NFT pathology, further underscore either 1) that other disease processes specific to the temporal lobe are present and are associated with dementia clinically presenting as high likelihood of Alzheimer's disease, or 2) the assessment of NP and NFT pathology by focal regional counts inadequately measures this marker of Alzheimer's disease.

However, it is also possible that the residual elevation in GFAP could be an artifact due to the inability to obtain NP and NFT counts in the same tissue utilized for GFAP quantification, though this seems unlikely. These data further suggest that vascular mediated pathology processes, or other co-existing pathologies, underlying the clinical categorization of likely vascular dementia, may be associated with active and ongoing astrogliosis throughout the brain.

Only in the occipital lobe does age at death appear to account for additional measures of variation in astrogliosis above-and-beyond NP and NFT pathology and clinical dementia information. A residual relation with age at death would suggest that chronic active disease processes are occurring which are inadequately characterized by neurodegenerative diseases associated 1) with NP and NFT pathology and 2) by the various sources of information used to create the clinical dementia categories and the CASI score. Neuropathological processes affecting visual image detection and associative spatial processing may be occurring--processes mediated in the occipital lobe which are not measured adequately by NP and NFT pathology and by cognitive-based clinical assessments of dementia.

Because the actual ages at death in this cohort are limited between 76 and 98 years, it *cannot* be inferred that the pathology and clinical assessments of this study effectively account for all disease processes associated with age-related astrogliosis. Certainly within the context of this study, age relations with parietal and temporal GFAP concentrations are attenuated to the point of statistical non-significance with the addition of information about NP and NFT pathology as well as clinical dementia. This would underscore that some aspects of relations between age and quantitative GFAP brain tissue concentration in these brain regions are mediated by age-related progressive neurodegenerative disease and attendant neuropathology.

In addition to the relatively narrow age range of study participants, the findings of this study are potentially limited by the homogeneity of ethnicity and gender and thus generalizations to women and other ethnic groups is reduced. However, it is unlikely that the underlying cellular and molecular mechanisms leading to dementia would be functionally different. There is also the potential that those participating in the autopsy component of the study are not fully representative of all participants, however a significant bias is unlikely since the focus was on a biochemical measure. The strengths of this study lie in it being a population-based longitudinal study design; the utilization of a standardized instrument to assess cognitive function; the use of uniform criteria for the neurological assessment of dementia; and a sensitive, quantitative measure of GFAP.

Dementia is a rapidly growing public health concern. Alzheimer's disease has been labelled as the singularly most important cause, and accordingly is an object of intense research. There is significant debate as to whether various histopathological and biomolecular characteristics attributed to this disease are sufficient to characterize the underlying derangements that ultimately lead to clinical dementia [42,43]. Indeed, it is likely that multiple processes related to vascular pathology [44] as well as the underlying processes of Alzheimer's disease operate conjointly in any given patient identified as cognitively impaired. Thus, the present data underscore the need to look for other neuropathological features, at both the cellular and molecular level, that may be contributing to the demented condition.

ACKNOWLEDGEMENTS

This work was supported in part by National Institute on Aging contract NO1-AG-4-2149; the United States Department of the Army grant DAMD17-98-1-8621, the Office of Research and Development Medical Research Service, Department of Veterans Affairs, and a contract from the National Institute of Occupational Safety and Health.

The information contained in this article does not necessarily reflect the position or the policy of the Government, and no official endorsement should be inferred.

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Figure 1. Scatterplots illustrating the relationship between GFAP levels and CASI scores in the frontal, occipital, parietal, and temporal cortices. Different symbols distinguish the various clinical diagnostic categories.

Table 1. Mean age, CASI score, and GFAP in each brain region by clinical classification.

				GFAP in μg/mg total protein (95% CI)[†]			
	N	Age (SD)	CASI (95% CI)	Frontal Lobe	Occipital Lobe	Parietal Lobe	Temporal Lobe
Likely AD	20	88.3 (6.4)***	31.7 (24.0, 39.5)***	12.3 (10.0, 15.2)	8.8 (7.0, 11.2)*	12.8 (9.8, 16.5)**	23.2 (17.0, 31.7)***
Likely MD	26	87.3 (4.7)***	40.8 (34.0, 47.7)***	11.8 (9.8, 14.2)	7.7 (6.2, 9.5)	10.4 (8.3, 13.1)	17.8 (13.5, 23.4)**
Likely VsD	22	86.5 (5.0)**	30.3 (22.9, 37.7)***	13.1 (10.7, 16.0)	8.6 (6.8, 10.7)*	9.0 (7.1, 11.6)	14.3 (10.6, 19.2)
Mild CI	55	86.2 (5.3)***	67.4 (62.7, 72.1)***	10.0 (8.8, 11.4)	7.0 (6.1, 8.0)	7.8 (6.7, 9.1)	10.8 (8.9, 13.0)
No Dementia	55	82.6 (4.9)	87.7 (83.0, 92.4)	10.4 (9.1, 11.8)	6.1 (5.3, 7.1)	7.8 (6.6, 9.1)	10.7 (8.8, 12.9)
No Classification	26	84.7 (4.7)		11.7 (9.7, 14.0)	8.0 (6.5, 9.8)	8.8 (7.0, 11.1)	15.9 (12.1, 20.8)*

* $p<0.05$, ** $p<0.01$, *** $p< 0.001$ relative to No Dementia group

[†] Mean GFAP levels are back calculated by exponentiation from the natural log transformed values used in the analyses

Table 2. Regression coefficients, R-square values, and mean GFAP differences derived from univariate and multivariate modelling of ln[GFAP] in each brain region.

		Frontal Lobe		Occipital Lobe		Parietal Lobe		Temporal Lobe	
Univariate	**Variable**	**Coefficient**	**R^2**	**Coefficient**	**R^2**	**Coefficient**	**R^2**	**Coefficient**	**R^2**
	Plaques	0.0156	0.0126	0.0366***	0.0523	0.0279*	0.0264	0.0472***	0.0786
	Tangles	0.0029	0.0007	0.0184**	0.0451	0.0252***	0.1063	0.0142***	0.0789
	Age	0.0100	0.0126	0.0232***	0.0521	0.0166*	0.0218	0.0197*	0.0201
	CASI	-0.0040**	0.0511	-0.0058***	0.0858	-0.0072***	0.1017	-0.0095***	0.1222
	Dementia Status		0.0387		0.0565		0.0698		0.1274
	Categories	**Mean Difference†**		**Mean Difference**		**Mean Difference**		**Mean Difference**	
	Likely AD	1.94		2.72**		4.99**		12.56***	
	Likely MD	1.43		1.57		2.63*		7.12**	
	Likely VsD	2.75		2.46*		1.28		3.62	
	Mild CI	-0.33		0.86		0.025		0.12	
	Unclassified	1.33		1.88*		1.05		5.20*	
Multivariate	**Variable**	**Coefficient**	**R^2**	**Coefficient**	**R^2**	**Coefficient**	**R^2**	**Coefficient**	**R^2**
Model 1			0.0126		0.0694		0.1083		0.1002
	Plaques	0.0155		0.0273*		0.0082		0.0299*	
	Tangles	0.0003		0.0124		0.0238***		0.0090*	
Model 2			0.0581&&		0.1153&&		0.1538&&		0.1756&&&
	Plaques	0.0113		0.0187		0.0039		0.0203	
	Tangles	-0.0048		0.0089		0.0183		0.0076	
	CASI	-0.0039**		-0.0045**		-0.0052**		-0.0076***	
Model 3			0.0479		0.1024		0.133		0.1823&&
	Plaques	0.0137		0.0224		0.0066		0.0245	
	Tangles	-0.0027		0.0114		0.0200***		0.0066	
	Dementia Status								
	Categories	**Mean Difference†**		**Mean Difference**		**Mean Difference**		**Mean Difference**	
	Likely AD	1.83		1.58		2.52		9.34***	
	Likely MD	1.33		1.18		2.15		6.49**	
	Likely VsD	2.57		2.21*		1.11		3.54	
	Mild CI	-0.43		0.83		0.13		0.52	
	Unclassified	1.23		1.69		1.01		4.96*	

Model 1: ln[GFAP] = Tangles + Plaques
Model 2: ln[GFAP] = Tangles + Plaques + CASI Score
Model 3: ln[GFAP] = Tangles + Plaques + Dementia Status

Significant coefficients or mean differences are indicated by * = $p<0.05$, ** = $p<0.01$, *** = $p< 0.001$
Significant differences relative to Model 1 are indicated by && = $p<.01$, &&& = $p < .001$.
† Differences in mean GFAP levels relative to the No Dementia group and are back calculated by exponentiation from natural log transformed values used in the analyses

APPENDIX P

Request for alterations to statement of work for DAMD 17-98-1-8621

TABLE: PESTICIDE LEVELS IN BRAIN TISSUE

batch	sample	Mass	Lipids	%Lipids	Surr-198	a-BHC	HCB	b-BHC	g-BHC	heptachlor	aldrin	isobenzan	hepox-b	oxychlordane	g-chlordane	endosulfan1	a-chlordane	t-nonachlo
QL						1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.
1	MB-1	0.08000			87%	nd	nd	nd	nd	nd	nd	nd	nd	nd	3.92	nd	nd	nd
1	MC-1				93%	89%	112%	95%	79%	67%	88%	75%	83%	81%	86%	83%	88%	90
1	2160A-27	0.08800	0.0050	5.6%	115%	nd	nd	nd	nd	nd	nd	nd	nd	5.93	4.54	nd	nd	5.
1	2160A-7	0.09800	0.0046	4.7%	109%	nd	nd	nd	nd	nd	nd	nd	nd	4.14	5.61	nd	2.85	4.
1	2422G-27	0.10400	0.0057	5.5%	113%	nd	nd	1.25	nd	nd	nd	nd	nd	2.78	4.60	nd	1.30	3.
1	2422G-7	0.08800	0.0034	3.8%	110%	nd	nd	nd	nd	1.56	nd	nd	nd	nd	5.61	nd	1.47	3.
1	9828D-27	0.08600	0.0053	6.1%	111%	nd	nd	nd	nd	nd	nd	nd	1.75	nd	4.17	nd	nd	2.
1	9828D-7	0.08500	0.0022	2.5%	114%	nd	nd	nd	nd	nd	nd	nd	nd	nd	5.25	nd	nd	1.
2	MB-2	0.08000			99%	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
2	MC-2				96%	89%	99%	95%	85%	42%	90%	61%	96%	114%	83%	93%	88%	96
2	1179I-18	0.11320	0.0063	5.6%	100%	nd	nd	1.45	nd	nd	nd	nd	nd	7.66	nd	nd	nd	4.
2	Du1179I-18	0.11320			99%	nd	nd	1.47	nd	nd	nd	nd	nd	7.34	nd	nd	nd	4.
2	1179I-8	0.07380	0.0022	3.0%	104%	nd	nd	nd	nd	nd	nd	nd	nd	4.22	nd	nd	nd	3.
2	2308E-27	0.05300	0.0026	4.8%	100%	nd	nd	nd	nd	nd	nd	nd	nd	nd	8.38	nd	2.55	nd
2	2308E-7	0.08190	0.0028	3.4%	96%	nd	nd	2.11	nd	2.49	nd	nd	nd	nd	6.51	nd	nd	nd
2	3085B-26	0.08370	0.0054	6.4%	96%	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
2	3085B-6	0.08670	0.0031	3.5%	94%	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
2	7726F-27	0.08130	0.0034	4.2%	99%	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.
2	7726F-7	0.08550	0.0042	4.9%	ns	nd	nd	nd	nd	nd	nd	nd	nd	2.11	5.30	nd	nd	2.
2	8349G-27	0.08320	0.0042	5.1%	100%	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
2	8349G-7	0.07310	0.0028	3.8%	102%	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
3	MB-3	0.08000			88%	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
3	MC-3				94%	77%	77%	89%	79%	33%	79%	55%	98%	96%	85%	86%	88%	89
3	0076J-39	0.07840	0.0044	5.6%	96%	nd	nd	nd	nd	nd	nd	nd	nd	2.65	nd	nd	nd	nd
3	0076J-49	0.08540	0.0055	6.4%	90%	nd	nd	nd	nd	nd	nd	nd	nd	2.26	nd	nd	nd	nd
3	1209D-27	0.09310	0.0067	7.1%	101%	nd	nd	nd	nd	nd	nd	nd	nd	4.14	nd	nd	nd	4.
3	1209D-7	0.08340	0.0027	3.2%	104%	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.
3	Du1209D-7	0.08340			96%	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.
3	2464E-27	0.09080	0.0029	3.1%	101%	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
3	2464E-7	0.09230	0.0017	1.8%	93%	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
3	3151C-38	0.10870	0.0038	3.5%	93%	nd	nd	1.25	nd	nd	nd	nd	nd	2.35	nd	nd	nd	1.
3	3151C-54	0.07860	0.0051	6.5%	100%	nd	nd	nd	nd	nd	nd	nd	nd	3.14	nd	nd	nd	2.
3	8535B-27	0.09520	0.0041	4.3%	87%	nd	1.38	3.31	nd	nd	nd	nd	nd	10.30	nd	nd	nd	7.
3	8535B-7	0.09120	0.0023	2.5%	95%	nd	nd	3.32	nd	nd	nd	nd	nd	5.19	nd	nd	nd	5.
3	9524J-27	0.08520	0.0043	5.0%	95%	nd	1.51	1.85	nd	nd	nd	nd	4.14	5.55	12.38	nd	3.93	6.
3	Du9524J-27	0.08520			101%	nd	1.86	nd	nd	nd	nd	nd	2.73	2.30	12.18	nd	5.04	7.
3	9524J-7	0.07470	0.0028	3.7%	93%	nd	nd	nd	nd	nd	nd	nd	nd	2.86	nd	nd	nd	2.
3	9763F-19	0.05110	0.0024	4.7%	90%	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
3	9763F-9	0.02370	0.0012	4.9%	ns	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

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sobenzan	hepox-b	oxychlordane	g-chlordane	endosulfan1	a-chlordane	t-nonachlor	dieldrin	DDE	endrin	endosulfan2	DDD	DDT	methoxych	mirex
1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	7.81	1.56
d	nd	nd	3.92	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
75%	83%	81%	86%	83%	88%	90%	80%	98%	77%	73%	93%	88%	71%	80%
d	nd	5.93	4.54	nd	nd	5.73	1.80	20.00	nd	nd	nd	nd	nd	nd
d	nd	4.14	5.61	nd	2.85	4.95	nd	16.14	1.90	nd	nd	nd	nd	nd
d	nd	2.78	4.60	nd	1.30	3.73	1.88	54.34	nd	nd	nd	nd	2.13	nd
d	nd	nd	5.61	nd	1.47	3.02	nd	42.68	nd	nd	nd	nd	nd	nd
d	1.75	nd	4.17	nd	nd	2.24	3.49	21.68	nd	nd	nd	nd	nd	nd
d	nd	nd	5.25	nd	nd	1.73	3.29	19.41	nd	nd	nd	nd	nd	nd
d	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
61%	96%	114%	83%	93%	88%	96%	86%	100%	9%	62%	89%	95%	84%	92%
d	nd	7.66	nd	nd	nd	4.39	nd	49.65	nd	nd	nd	nd	nd	nd
d	nd	7.34	nd	nd	nd	4.41	nd	48.59	nd	nd	nd	nd	nd	nd
d	nd	4.22	nd	nd	nd	3.11	nd	33.30	nd	nd	nd	nd	nd	nd
d	nd	nd	8.38	nd	2.55	nd	nd	16.96	nd	nd	nd	nd	nd	nd
d	nd	nd	6.51	nd	nd	nd	nd	11.78	nd	nd	nd	nd	5.27	nd
d	nd	nd	nd	nd	nd	nd	nd	7.92	nd	nd	nd	nd	nd	nd
d	nd	nd	nd	nd	nd	nd	nd	5.79	nd	nd	nd	nd	4.38	nd
d	nd	nd	nd	nd	nd	1.82	nd	7.41	nd	nd	nd	nd	nd	nd
d	nd	2.11	5.30	nd	nd	2.06	nd	7.68	nd	nd	nd	nd	nd	nd
d	nd	nd	nd	nd	nd	nd	nd	3.48	nd	nd	nd	nd	nd	nd
d	nd	nd	nd	nd	nd	nd	nd	3.26	nd	nd	nd	nd	7.44	nd
d	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
55%	98%	96%	85%	86%	88%	89%	90%	91%	33%	67%	97%	82%	93%	89%
d	nd	2.65	nd	nd	nd	nd	nd	5.42	8.47	nd	nd	nd	9.67	nd
d	nd	2.15	nd	nd	nd	nd	nd	5.85	4.44	nd	nd	nd	6.14	nd
d	nd	4.14	nd	nd	nd	4.27	nd	39.58	nd	nd	nd	nd	nd	nd
d	nd	nd	nd	nd	nd	2.20	nd	25.92	nd	nd	nd	nd	3.68	nd
d	nd	nd	nd	nd	nd	1.62	nd	25.28	nd	nd	nd	nd	nd	nd
d	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
d	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
d	nd	2.35	nd	nd	nd	1.70	nd	27.38	3.41	nd	nd	nd	3.74	nd
d	nd	3.14	nd	nd	nd	2.84	nd	36.30	5.48	nd	nd	1.79	10.20	nd
d	nd	10.30	nd	nd	nd	7.49	nd	25.01	nd	nd	nd	1.43	6.61	nd
d	nd	5.19	nd	nd	nd	5.25	nd	19.78	nd	nd	nd	nd	5.10	nd
d	4.14	5.55	12.38	nd	3.93	6.50	13.24	93.66	1.54	nd	2.14	nd	5.13	nd
d	2.73	2.30	12.18	nd	5.04	7.90	3.24	85.55	nd	nd	nd	nd	nd	1.53
d	nd	2.86	nd	nd	nd	2.85	nd	61.95	nd	nd	nd	nd	nd	nd
d	nd	nd	nd	nd	nd	nd	nd	2.66	3.64	nd	nd	nd	12.78	nd
d	nd	nd	nd	nd	nd	nd	nd	nd	11.39	nd	nd	nd	26.05	nd

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LEADING ARTICLE

Drugs Aging 2001; 18 (11): 797-806
1170-229X/01/0011-0797/$22.00/0

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Current Evidence for Neuroprotective Effects of Nicotine and Caffeine Against Parkinson's Disease

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Abstract

Parkinson's disease (PD) is the second most common neurodegenerative disorder affecting 1 to 3% of individuals over the age of 65 years. While effective therapy exists for treating the bradykinesia, rigidity and tremor associated with the disease, the cause is unknown. There is no treatment available to prevent or slow the progressive neuronal loss in the substantia nigra and associated decreased levels of dopamine in the striatum that underlie the cardinal features of the disease.

Both retrospective and prospective epidemiological studies have consistently demonstrated an inverse association between cigarette smoking and PD, leading to theories that smoking in general and nicotine in particular might be neuroprotective. Nicotine has been shown in animals to stimulate the release of dopamine in the striatum, and to preserve nigral neurons and striatal dopamine levels in laboratory animals with lesioned nigrostriatal pathways.

Coffee and caffeine consumption have also been shown in epidemiological studies to be inversely related to PD risk. Caffeine is an adenosine A_{2A} receptor antagonist that enhances locomotor activity in animal models of parkinsonism. Theophylline, a related compound that has A_{2A} receptor blocking properties, has been shown in one small trial to improve motor function in patients with PD.

Recently, potent and highly selective A_{2A} receptor antagonists have been developed that have demonstrated improvement in motor function in animal models of parkinsonism. Exciting findings are emerging that demonstrate attenuation of dopaminergic neurotoxicity with caffeine and other adenosine receptor antagonists in mice given the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), suggesting that these compounds may be neuroprotective.

Evidence for the neuroprotective potential of nicotine and caffeine is compelling, but further work is needed before testing these and related compounds in clinical trials for both individuals at high risk of developing PD and those with early, untreated disease.

Parkinson's disease (PD) is a progressive neurodegenerative brain disorder characterised by bradykinesia, rigidity with cogwheeling and rest tremor. Postural instability is another cardinal feature that generally occurs later in the course of the disease. Underlying the movement disorder of PD is a selective loss of dopamine-producing neurons in the pars compacta region of the substantia nigra resulting in low levels of the neurotransmitter dopamine in the striatum. This neurochemical deficit is the basis for treatment of patients with PD with levodopa, and a good clinical response to levodopa is considered supportive of a diagnosis of PD.[1,2] The cause of PD is unknown and currently there is no treatment that prevents or arrests the disease process.

Approximately 1 to 3% of individuals over the age of 65 years have PD.[1] Despite effective symptomatic therapy, the disease progresses inexorably, although at variable rates, and patients with PD eventually become severely debilitated requiring total care. Estimates of annual direct costs related to PD in the US range from 7.1 to 25 billion American dollars per year.[3,4] Additionally, there is substantial loss of productivity – 15% of patients with PD are unable to work after 5 years and 80 to 90% are unable after 9 years.[3,4]

Among the most important areas of research in PD is the search for environmental determinants or medications that prevent, arrest or slow the disease process. To date, only the monoamine oxidase (MAO) B inhibitor, selegiline (deprenyl), has shown promise as a neuroprotective agent for PD, but studies remain inconclusive.[5,6]

Intriguing findings from case-control and longitudinal studies suggest that coffee and cigarette smoking may have neuroprotective actions against PD. Biologically plausible mechanisms exist for both substances. Animal models of parkinsonism demonstrate that caffeine and nicotine prevent neurotoxin-induced neuronal loss in the substantia nigra and/or reduction of dopamine in the striatum. This review will examine epidemiological evidence that cigarette smoking and caffeine consumption are inversely related to PD risk, explore possible biological mechanisms for neuroprotection and discuss therapeutic implications.

1. Smoking and Nicotine

1.1 Epidemiological Evidence for Inverse Association of Smoking and Parkinson's Disease (PD)

One of the strongest and most consistent observations in epidemiological studies of PD is the inverse association with cigarette smoking. It was first noted over 40 years ago in mortality studies that risk of death from PD was lower in smokers than nonsmokers.[7] Subsequently, numerous case-control studies have found smoking to be significantly more common in controls than in PD cases.[8-10] In fact, this relationship has been found in 34 of 35 separate studies according to a recent review.[11] Odds measurements from these studies combined approximated 0.5, indicating that patients with PD are half as likely to have a history of smoking as individuals without PD. Prospective studies such as the longitudinal Honolulu Heart Program have produced similar findings. In the latter study, individuals that had ever smoked had a significantly reduced risk of PD [relative risk = 0.44, 95% confidence interval (CI) = 0.26-0.75] in a model including age, coffee drinking, and alcohol consumption.[12] A dose-response relationship between pack-years of smoking and PD risk was apparent. A dose-response effect was also seen in a recent case-control study where the inverse association of smoking and PD was greater for heavy smokers [odds ratio (OR) = 0.08; 95% CI = 0.01-0.62] than light smokers (OR = 0.59; 95% CI = 0.23-1.53).[13] Studies in Europe,[14,15] Asia,[16,17] and South America,[18] all showing an inverse association between smoking and PD, are evidence of the consistency of this finding across diverse geographical and ethnic groups.

The explanation for this finding is controversial. Some have argued that reluctance to smoke is characteristic of a premorbid PD personality.[11] However, plausible explanations exist for a direct effect of smoking on PD risk, either through symptomatic

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relief of early PD symptoms or neuroprotection. Cigarette smoke is known to contain thousands of chemical substances, any one of which could be responsible for this effect.[19] The constituent in tobacco smoke most studied in this regard is nicotine.

1.2 Direct Pharmacological Effects of Nicotine

Nicotinic acetylcholine receptors (nAChR) are ligand-gated ion channels that exist in most regions of the brain and are present on nigrostriatal dopaminergic neurons.[20,21] Their precise role(s) in the CNS has not been determined. However, stimulation of these receptors leads to acetylcholine release, modification of CNS neuronal excitability and regulation of release of other neurotransmitters including dopamine.[22]

Dopamine output measured by *in vivo* microdialysis is increased in the striatum of normal rats following acute subcutaneous nicotine administration.[23] Interestingly, locomotor activity in normal rats is initially depressed then increased following administration of nicotine. With repeated doses tolerance to the depressant action develops, but the stimulant effects persist.[24] Similar effects have been noted following nicotine administration in mice with parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).[25]

Improvement in PD symptoms with nicotine intake has also been studied in humans. One study of six patients with early-onset PD showed that smoking reduced tremor, rigidity, bradykinesia and gait disturbances. The effects lasted for 10 to 30 minutes. Nicotine chewing gum had a lesser effect.[26] Another study of two elderly patients with PD using a single-subject, placebo-control reversal design demonstrated improvement in symptoms of tremor, disorganised thinking and bradykinesia after administration of nicotine in gum and transdermal patches.[27] Preliminary results from another study of chronic administration of nicotine by transdermal patch revealed improvement in motor functioning in patients with PD.[28] Three other small studies showed either no change in PD symptoms with nicotine chewing gum,[29] or acute worsening of PD symptoms with transdermal nicotine administration[30] or cigarette smoking.[31] Nicotine in the formulations and dosage from the trials cited above was generally well tolerated by patients, although nausea and vomiting occurred in a small number of cases.[28,29]

The disparity in results regarding the pharmacological effect of nicotine may be because of differing routes and schedules of administration. In one study showing worsening of symptoms a high dose of nicotine was used.[30]

Clearly, the harmful effects of smoking including lung cancer and cardiovascular disease far outweigh the potential benefits smoking might have for patients with PD. More studies with larger sample sizes are needed to assess the usefulness of nicotine and nicotine agonists for symptomatic therapy of PD in the future.

1.3 Evidence for a Neuroprotective Effect of Nicotine

Administration of nicotine in experimental animal models of PD has demonstrated that nicotine can counteract dopaminergic cell loss. In rats with partial unilateral mesodiencephalic transection (a commonly used model of parkinsonism), long term infusion of nicotine delivered by subcutaneously implanted osmotic pumps partially prevented the lesion-induced reduction in number of nigral dopamine neurons but did not affect other populations of neurons or non-neuronal cells.[32] Also, exposure to tobacco smoke prior to lesioning of dopaminergic neurons with MPTP has been shown to reduce the resulting decrease in striatal dopamine levels in mice.[33]

However, not all studies of nicotine in experimental models of PD have found a beneficial effect.[34] Some researchers have found that nicotine potentiated MPTP-induced toxicity in mice.[35] The explanation for these inconsistent results may be related to the dose schedule of nicotine and extent of the nigral lesion. More recent studies seem to support these ideas. Nicotine administered subcutaneously to rats at intervals before and after lesioning

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of the nigra with 6-hydroxydopamine administration counteracted striatal dopamine loss. This effect was not present when nicotine was given as a single dose at any time or when a high dose of 6-hydroxydopamine (10 *vs* 6μg) that resulted in a more extensive lesion was given.[36] Another study found the neuroprotective effects of long term nicotine therapy in 6-hydroxydopamine–treated rats to be dependent on nicotine dose with low, but not high, doses inhibiting neuronal degeneration.[37]

1.4 Possible Mechanisms for Protection of Nigral Dopaminergic Neurons by Nicotine

Nicotine pretreatment prevents glutamate-induced neurotoxicity in several types of neuronal cultures[38-42] including striatal neurons.[43] Recently a study of neurotoxicity induced by methyl-4-phenylpyridine (MPP+), a metabolite of MPTP, in mesencephalic neurons found that nicotine attenuated the expected decline in number of dopaminergic cells. This attenuation was blocked by a nicotine receptor antagonist, suggesting the effect was mediated by the nAChR.[44]

Oxidative stress has been linked to the neurodegeneration that occurs in patients with PD.[45] Hydrogen peroxide is produced when dopamine is metabolised by MAO and is present at relatively high levels in the substantia nigra.[46] Free radicals with toxic effects that can cause neuronal injury and death are generated by the iron-mediated catalysis of hydrogen peroxide in the Fenton reaction. Normally, natural antioxidants counteract the toxic effects of free radicals. Oxidative stress is said to exist when there is production of free radicals in excess of the detoxifying capabilities of the organism's natural antioxidants.[45,47]

Nicotine may act as an antioxidant by inhibition of the Fenton reaction as demonstrated *in vitro*.[48,49] Additionally, smoking may reduce free radical formation via a reduction of brain MAO levels. In a study of human volunteers, positron emission tomography of the brain showed a 40% decrease in the level of MAO B in smokers relative to non-smokers or past smokers.[50] The constituent in tobacco smoke responsible for this effect is unknown.

Studies in rats and mice have demonstrated that nicotine induces increased mRNA levels for both fibroblast growth factor-2 (FGF-2) and brain-derived neurotrophic factor, resulting in higher levels of these neurotrophins in striatum[51] and ventral midbrain.[52] These growth factors have been shown to stimulate dopaminergic neuron survival *in vivo*.[51,53] In the parkinsonian model of mice treated with MPTP, intracerebral administration of FGF-2 induces recovery of dopaminergic function.[54] Autopsy studies have shown that FGF-2 levels are severely reduced in substantia nigra in brains affected by PD.[55] For both neurotrophins, increased production induced by nicotine is evidently mediated by activation of nAChR because it can be blocked by a nicotinic receptor antagonist.[51,52] Recently, an nAChR agonist, epibatidine, has been shown to increase FGF-2 levels in the rat brain.[56]

Another possible neuroprotective mechanism of nicotine that could be mediated by nAChR is upregulation of cerebral blood flow. Studies in animals reveal that cholinergic neurons located in the basal forebrain region largely influence cerebral blood flow.[57,58] In one study, intravenous nicotine administered to rats significantly increased cortical cerebral blood flow independent of blood pressure and the effect was blocked by a nicotinic receptor antagonist.[59] Nicotine also induces increased cerebral glucose utilisation in rats in numerous brain regions[60] including substantia nigra.[61]

Studies in humans have shown cigarette smoking to increase cerebral vessel blood flow measured by ultrasonic Doppler.[62,63] In one study there was a dose effect, with cigarettes containing a higher level of nicotine producing a greater effect on cerebral blood flow.[62] Nicotine administered to healthy adults who smoke has also been shown to increase regional cerebral blood flow to cortical and subcortical regions as measured by positron emission tomography.[64]

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2. Coffee, Caffeine and Other Adenosine A_{2A} Receptor Antagonists

The stimulant effect of the xanthine derivative caffeine is well established and thought to be due, in part, to enhancement of dopamine neurotransmission.[65,66] Animal experiments in the mid-1970s indicated that caffeine potentiated the effect of levodopa and dopamine receptor agonists.[67] These findings provided the basis for human trials of caffeine administered with the dopamine agonists piribedil and bromocriptine and with levodopa to study participants with PD.[68,69] The results of these trials were negative, but the number of study participants in both trials was small (six in each) and duration of treatment was 2 weeks or less.

2.1 Epidemiological Evidence for Inverse Association of Coffee and Caffeine Consumption with PD

One of the earliest epidemiological studies examining the association of coffee consumption with PD found that a greater percentage of patients with PD than of the control group were not coffee drinkers.[9] This was speculated to be a consequence of the disease. Two case-control studies found that premorbid coffee consumption was significantly less in individuals with PD compared with the control group. In both studies there was an inverse dose-response relationship between coffee consumption and PD.[70,71] Other studies have not found a significant association.[72] While most of these studies attempted to assess coffee drinking habits prior to disease onset, it remains possible that current dietary habits biased recall of prior coffee drinking. If having PD causes patients to drink less coffee, they may have a tendency to under-report past coffee consumption.

Prospective studies aim to avoid recall bias by assessing coffee consumption in healthy study participants and following them for incident PD. The Honolulu Heart Program is a longitudinal study of aging and neurodegenerative disorders in Japanese-American men participating since 1965.[73] Coffee consumption was measured at two separate examinations in 1965 and 1971. Median follow-up from baseline examination in 1965 was 27 years (range 1 to 30) and median interval between the examination in 1965 and PD onset was 16.6 years (range 2 to 30 years). Cases of PD were identified through medical and death records, and examination by neurologists. Age-adjusted incidence of PD declined consistently with increased amounts of coffee consumed for both examinations analysed separately. The age-adjusted incidence of PD for non-coffee drinkers (assessed in 1965) was 10.5 per 10 000 person-years while the incidence for those drinking 28 ounces (approximately equivalent to 800ml or 7 small cups) of coffee or more per day was 1.7 per 10 000 person years ($p < 0.001$). After adjusting for age and cigarette smoking the relative hazard for nondrinkers versus those drinking 28 ounces or more of coffee was 5.1 (95% CI = 1.8-14.4).[73] This relationship was also observed for total caffeine consumption, which included coffee and non-coffee dietary sources [relative hazard for lowest versus highest quintile was 5.1 (95% CI = 2.1-12.3)] and for caffeine from non-coffee sources [relative hazard for lowest versus highest quintile was 2.7 (95% CI, 1.4-5.4)]. No other nutrients in coffee including niacin, milk and sugar were associated with PD.

Recently, investigators from the prospective Nurses Health Study and the Health Professionals Follow-Up Study reported regular caffeine consumption to be protective against incident PD. The relationship was strongest among men, for whom a dose response pattern was demonstrated. Importantly, both coffee and tea appeared to be protective but not decaffeinated coffee. For women the relationship was U-shaped with moderate intake of caffeine being associated with the lowest risk of PD.[74]

Together, these studies provide strong evidence that coffee consumption is associated with a lower risk of future development of PD and that caffeine is the responsible constituent. Observational studies, however, cannot provide proof that caffeine is protective. Caffeine may be a surrogate for an un-

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recognised factor responsible for the association. For example, persons destined to develop PD may be intolerant to the effects of caffeine. Alternatively, caffeine may provide beneficial symptomatic effects in early PD masking symptoms.

Recent research using animal models of parkinsonism has provided support for both symptomatic and neuroprotective roles of caffeine and related compounds, and has offered plausible biological mechanisms for these effects.

2.2 Pharmacological Effects of Caffeine

Caffeine is a methylxanthine derivative CNS stimulant thought to enhance dopamine neurotransmission by acting as an adenosine receptor antagonist.[65,66,75,76] Adenosine receptors are highly concentrated in the caudate nucleus, putamen, nucleus accumbens, globus pallidus and olfactory tubercle. Among four subtypes of adenosine receptors, the A_{2A} subtype has attracted the most attention in PD.[76,77]

In the striatum, A_{2A} receptors are co-localised with dopamine D_2 receptors in γ-aminobutyric acid (GABA)-ergic neurons.[75] Stimulation of A_{2A} receptors decreases dopamine neurotransmission[78] and decreases affinity of D_2 agonists for D_2 receptors.[76,79] In experimental animal models, stimulation of adenosine receptors produces decreased locomotor activity,[65,80-82] while adenosine receptor blockers have the opposite effect.

In rodent models of PD with unilateral lesions of the nigra induced by 6-hydroxydopamine, caffeine and other adenosine receptor antagonists induce contralateral rotation behaviour indicating anti-parkinsonian effects.[76] Recently, highly potent A_{2A} receptor antagonists have been developed. One of these, KW-6002, has been shown to ameliorate hypokinesia induced by the dopamine-depleting agent reserpine and the neurotoxin MPTP.[83,84] This compound has also been reported to improve motor deficits in primates with MPTP-induced parkinsonism.[85,86] These experiments demonstrate beneficial symptomatic effects of adenosine receptor antagonists on motor function in animal models of parkinsonism.

Pharmaceutical trials in humans are beginning to support this. One open label trial of theophylline, a methylxanthine A_{2A} receptor antagonist, given to 15 patients with PD reported significant improvement in motor function as measured by the Unified Parkinson's Disease Rating Scale.[87] Another small trial reported that theophylline was associated with longer 'on' time in patients with advanced PD treated with levodopa/carbidopa.[88]

The methylxanthines including caffeine are generally well tolerated. Elevated heart rate and tremor may accompany the stimulant effects even with modest doses. Excessive consumption of caffeine causes anxiety, agitation, insomnia, tachycardia and gastrointestinal upset. Abrupt withdrawal of caffeine in long term users can be associated with withdrawal symptoms such as fatigue, drowsiness, headaches and nausea.[65]

2.3 Evidence for a Neuroprotective Effect of A_{2A} Receptor Antagonists

In addition to acute beneficial symptomatic effects, recent work suggests that A_{2A} receptor antagonists may be neuroprotective. Evidence first arose in animal models of stroke. Selective A_{2A} receptor antagonists reduce stroke-related brain injury in rodent models of cerebrovascular ischaemia.[89-91] Recently, inactivation of the A_{2A} receptor in A_{2A} knock-out mice has been shown to protect the brain from transient focal ischaemia.[92] The mechanism is unknown but may be related to diminished release or blocked effect of excitatory amino acids following ischaemia.[93]

Studies to evaluate the potential for A_{2A} receptor antagonists to arrest neurodegenerative processes leading to dopaminergic neuron loss in substantia nigra are beginning. Recently, findings have been published demonstrating attenuation of dopaminergic lesions in the MPTP model of parkinsonism in mice. Mice pretreated with caffeine as well as A_{2A} receptor-specific antagonists all showed reduced MPTP-induced losses of striatal dopamine and dopamine transporter. Knockout mice lacking the A_{2A} receptor also demonstrated decreased MPTP-induced neurotoxicity.[94] This work sup-

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ports the possibility that A_{2A} receptor blockade confers a neuroprotective effect in PD. The mechanism of this effect as well as relevance of this model to humans are uncertain.

2.4 Potentiation of Neuroprotective Action of Dopamine Agonists

Evidence is growing that dopamine agonists used in the symptomatic treatment of PD are neuroprotective.[95] A recent study examined neuroprotective effects of dopamine agonists against glutamate-induced neurotoxicity in cultured rat mesencephalic neurons. D_2 receptor agonists (bromocriptine and quinpirole), but not a D_1 receptor agonist, blocked glutamate-induced neurotoxicity when cells were incubated prior to exposure to glutamate.[95] A_{2A} receptor antagonists may enhance this effect by increasing affinity of dopamine agonists for D_2 receptors.[79,95] Combination therapy coupling dopamine agonists with A_{2A} receptor antagonists may be a strategy for preventing dopaminergic neuron degeneration in patients with PD.

3. Conclusions

Ongoing trials are evaluating acute effects of adenosine receptor antagonists such as KW-6002 and nicotine on symptoms of PD. New potent, specific antagonists of the A_{2A} receptor are being developed.[93] Safety, tolerability and dosage range recommendations will be established for these medications in the management of symptoms in patients with PD.

The ultimate goal, however, must be to find medications that prevent or arrest progression of PD. Clinical trials of candidate neuroprotective agents could be done with untreated patients in the earliest stages of PD or in individuals at high risk for developing PD. Future use of genetic or imaging bio-markers may make it possible to screen large groups of asymptomatic individuals to identify those at high risk for developing the disease. Endpoints in such trials may be defined as onset of PD or changes in dopaminergic function measured by functional imaging tools such as [^{18}F]-dopa positron emission topography and I^{123} 2-β carboxymethoxy-3-β(4-iodopheryl) tropane single photon emission computed tomography (B-CIT SPECT) that are indicators of nigrostriatal degeneration.[96,97]

Before committing humans to lengthy, potentially risky and expensive trials, medications to be tested must be selected based on sound biological plausibility, strong epidemiological evidence and proven efficacy in animal models. Nicotine receptor agonists and adenosine receptor antagonists are promising neuroprotective agents. However, the evidence for a protective effect of nicotine in rodent models of parkinsonism is inconsistent. This may be related to variations in method of administration or dose regimen. Primate models may be helpful in this area. The evidence for a neuroprotective effect of adenosine receptor antagonists is just emerging and needs confirmation. High priority should be placed on clarifying these remaining issues so that research can move forward to neuroprotective trials in humans.

Acknowledgements

The author's work cited in this paper was supported by US Department of the Army grant DAMD17-98-1-8621; National Institute on Aging contract N01-AG-4-2149; and Office of Research and Development Medical Research Service, Department of Veterans Affairs. The information contained in this article does not necessarily reflect the position or the policy of the government, and no official endorsement should be inferred.

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APPENDIX R

THE MEDICAL CLINICS OF NORTH AMERICA

Med Clin N Am 86 (2002) 455–476

The diagnosis and differential diagnosis of dementia

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Dementia is one of the most costly and disabling diseases associated with aging. The emotional impact of the disease on patients and families is devastating, and the cost to society is staggering. Annual costs for caring for a single patient with Alzheimer's disease (AD) are reported to be between $35,000 and $47,000, totaling more than $140 billion dollars per year in the United States assuming there are 4 million people with AD [1–3]. This figure is projected to rise as the proportion of the population older than the age of 65 years increases. As treatments for AD and other dementias that can extend the period individuals have reasonably good cognitive and physical functioning become available, the accurate and early diagnosis of these disorders becomes more crucial.

This article provides an overview of the bedside and clinic evaluation of patients with complaints of forgetfulness or other cognitive or behavioral disturbances and reviews distinguishing features of dementia and other conditions that may be confused with dementia. The laboratory and imaging evaluation of the patient with dementia and the various causes of dementia are described. Current clinical practice guidelines and practice parameters are reviewed as relevant for the primary care practitioner. For the most part, these guidelines are similar; however, there are important inconsistencies that are discussed.

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This work was supported by National Institute on Aging contract NO1-AG-4-2149, US Department of the Army grant DAMD17-98-1-8621, and Department of Veterans Affairs medical research funds. The information contained in this article does not necessarily reflect the position or the policy of the US government, and no official endorsement should be inferred.

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PII: S0025-7125(02)00009-3

Dementia is a clinical syndrome characterized by acquired impairment in multiple neuropsychologic and behavioral domains, including memory, language and speech, visuospatial ability, cognition (the ability to manipulate previously learned information), and mood/personality [4]. Most definitions, such as that of the fourth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) [5], require that the intellectual deficits be of sufficient severity to impair social or occupational functioning. These definitions necessarily draw an arbitrary line between no dementia and dementia. Patients most often pass through stages of intellectual decline that may or may not progress to dementia. These include cognitive deficits thought to occur with normal aging; mild cognitive impairment (MCI), a term used to recognize a transitional phase from normal aging to dementia; and early dementia. In practice, it is often difficult to recognize when a patient with memory complaints has a process that is likely to progress to further intellectual and functional decline. Because there is no diagnostic test to identify dementia, the clinician must rely on a careful and accurate history from the patient and a reliable informant as well as a good mental status examination.

As effective treatments are developed for the common causes of dementia, it is important to diagnose patients in the earliest phases of disease, perhaps even before affected individuals recognize that a memory problem exists. According to one recently published population-based study, approximately 20% of family informants failed to recognize memory problems in elderly subjects who were found to have dementia on a comprehensive standardized examination [6]. Another study found that more than 75% of patients in a primary care group practice found to have dementia on cognitive screening had no documentation of cognitive impairment in their medical record [7]. It is thus essential that clinicians inquire about cognitive and functional changes in their elderly patients and administer a brief mental status examination if changes exist so as to identify cognitive decline as early as possible.

Clinical evaluation of patients with memory complaints

History of cognitive impairment

Patients who complain of memory problems and those suspected to have memory problems should give a thorough and detailed history, taken from the patient as well as from a relative or close friend. Even in the early stages of dementia, such as AD or frontotemporal dementia, patients may be unable to recall important aspects of their medical history and may even deny or not recognize significant memory deficits because of lack of insight. Family members can provide information regarding the time and character of onset as well as the pattern of progression of the memory complaints. For example, the onset of AD is insidious, and the course is slowly progressive, whereas vascular dementia (VsD) may begin abruptly and be associated with a stepwise decline [5,8,9]. Although there are exceptions to these rules, they do

provide useful information for distinguishing these two common dementia syndromes. It is also helpful to inquire about life events temporally related to the onset of the memory complaints, such as hospitalization, stroke, head injury, new medications, or the loss of a spouse or other family member.

Inquiry should be made regarding ability to learn and recall new information. Examples include recall of recent conversations and events, telephone messages, short shopping lists, appointments, and use of new appliances or electronic devices. Patients may forget to pay their bills, become lost in familiar surroundings, lose items (eg, mail or keys), or exhibit repetitive behaviors (eg, asking the same question over and over). Standardized informant-based questionnaires regarding memory and cognitive decline may be administered by trained interviewers, providing a base for a more focused history taken by the primary physician. One of these tools is the Informant Questionnaire on Cognitive Decline in the Elderly [10,11].

History of behavioral disturbances

It should be recognized that memory impairment, although a hallmark of the dementia syndrome, is not always the presenting feature. Behavioral disturbances and decline in functional ability are often the triggers that lead family members to seek medical attention [6]. Behavioral disturbances include delusions, hallucinations, changes in mood (eg, depression), and changes in personality (eg, disinhibition, impulsivity, loss of interest in usual activities, excessive anxiety, uncharacteristic anger or agitation). Standardized assessments of behavioral complications are also available, such as the Neuropsychiatric Inventory [12] or the Behave-AD [13]. These assist the clinician in screening for a range of behavioral complications associated with dementia and in measuring the effect that these complications have on the caregiver's well-being.

History of functional impairment

An important component of the history is assessing the impact that the intellectual decline and behavioral disturbances are having on the patient's social and basic functioning ability. This may be a difficult task in the elderly, especially when physical ailments also contribute to inactivity or decline in functional ability. As a result, some knowledge of the patient's previous activities is necessary. Standardized instruments, such as the informant-rated Blessed Dementia Scale [14], the Functional Activity Questionnaire [15], or the Instrumental Activities of Daily Living Scale [16], may be used for functional assessment. These scales assess activities of daily living (personal maintenance activities essential for good health and well-being), including dressing, bathing, eating, and toileting, as well as instrumental activities of daily living (higher order skills required for independent living), including managing money, preparing meals, taking

medication, and performing household tasks. More detailed discussions of these topics are available in recently published guidelines and reviews [17–20].

A review of the patient's past medical and psychiatric history for conditions that may contribute to cognitive decline is always necessary. This should include questions regarding cardiovascular disease, remote head trauma, alcohol use, prescription and nonprescription medications, dietary supplements, and treatment for depression. Family history is important, because many dementing diseases have a familial component. Thorough reviews of systems and a general physical examination, including a detailed neurologic examination, are needed to identify clues to the diagnosis. A cardiac murmur or dysrhythmia along with focal neurologic signs may suggest a vascular etiology, whereas bradykinesia, rigidity, and tremor suggest one of the parkinsonian syndromes.

Mental status examination

The bedside mental status examination should include assessment of level of consciousness, orientation, attention, speech and language, recent and remote memory, cognition, visuospatial skills and mood/personality. Attention refers to the patient's ability to maintain focus on the appropriate stimulus while avoiding distraction from irrelevant stimuli. This may be tested by digit repetition or by subtracting serial 7's or 3's from 100. In general, attention is preserved until late in most dementias. Language assessment begins with listening to the patient's spontaneous verbal output and includes word list generation, confrontation naming, repetition, and comprehension. Aphasia may suggest dominant hemisphere disease; however, impoverished spontaneous verbal output with paraphasic errors (word substitutions) may also be an early indication of AD [21]. Recent memory may be tested by asking the patient to recall a list of words after 3 to 5 minutes. The minimum is three words; however, longer lists, such as eight words, allow the generation of a learning curve. During the recall phase, category or multiple choice cues may be given to determine if the words have been learned. These techniques can be helpful for differentiating the forgetfulness (ie, intact learning, impaired retrieval) of normal aging and certain subcortical dementia syndromes from the amnesia (ie, impaired learning and retrieval) of AD and other cortical syndromes [4]. Visuospatial function is tested at the bedside by asking the patient to copy two- and three-dimensional geometric shapes. Cognition or the ability to manipulate previously learned information includes assessment of abstracting ability by interpretation of similarities, proverbs, and mental arithmetic. Performance of these tasks is highly dependent on educational level. Executive function refers to judgment and motivation as well as planning, performing, and monitoring complex behaviors. Impairment in this area is characteristic of subcortical dementia syndromes. The reader is directed to several references with excellent descriptions of the mental status examination for more detail [4,22]. The bedside mental status examination

provides valuable qualitative information in multiple cognitive domains; however, the time and expertise to administer the full examination may make it impractical in the average primary care setting. Clinicians may prefer a brief standardized cognitive assessment instrument such as the Mini-Mental State Examination [23], the Cognitive Abilities Screening Instrument [24], the Alzheimer's Disease Assessment Scale [25], or the Neuropsychological Assessment of the Consortium to Establish a Registry for Alzheimer's Disease [26]. These all have the advantage of being quantitative and easy to administer. When used along with the other components of the history and examination, they are useful for determining if a patient has dementia. Because of the variable sensitivity and specificity in different populations and the effects of education on performance, a specific cutoff score cannot be the sole method of diagnosing dementia. Scores are useful for measuring change over time [18,19]. The Mini-Mental State Examination is the most commonly used cognitive screening instrument (Table 1). It has the advantages of being brief, easy to administer, and inclusive of multiple domains, including orientation, attention, language, memory, and visuospatial ability. Published normative data allow interpretation of scores according to the patient's age and education [27].

In addition to age and education, impairment in hearing and vision as well as cultural and language background may affect performance on cognitive testing [18,28]. Nonnative English speakers may have difficulty with

Table 1
Mini-mental state examination

	Maximum score
What is the (year)(season)(date)(day)(month)?	5
Where are we (state)(country)(town)(hospital)(floor)?	5
Registration	
Name three objects. Give 1 second to say each. Ask the patient all three after you have said them. Give 1 point for each correct answer. Repeat them until the patient learns all three count trials and record.	3
Attention and calculation	
Serial 7's. Give 1 point for each correct. Stop after five answers. Alternatively, spell "world" backwards.	5
Recall	
Ask for the three objects repeated previously. Give 1 point for each correct answer.	3
Language	
Names a pencil and watch.	2
Repeat the following: "No ifs, ands, or buts."	1
Follow a three-stage command: "Take a paper in your right hand, fold it in half, and put it on the floor."	3
Read and obey the following: CLOSE YOUR EYES.	1
Write a sentence.	1
Copy design [two overlapping pentagrams].	1

cognitive function tests given in English. Individuals relatively fluent in English may still perform at a higher level in their native language given the stress of cognitive function tests. Cognitive screening instruments translated into the native language should be used when available. Even when assessing cognition in the language most comfortable for the patient, culturally biased items on the screening test used may still affect performance, requiring interpretation of the test results within culture-specific norms [18,28].

Formal neuropsychologic testing may be necessary when the bedside assessment fails to differentiate between changes associated with normal aging and early dementia. These tests may also assist with narrowing the differential diagnosis of the dementia syndrome.

Diagnostic evaluation of dementia

Neuroimaging

Although the literature regarding indications for neuroimaging in the evaluation of dementia remains inconclusive, most dementia specialists suggest that a structural brain image be obtained for a newly diagnosed patient to assess cerebrovascular lesions, neoplasms, subdural hematomas, or hydrocephalus [17]. The recent report from the Quality Standards Subcommittee of the American Academy of Neurology on the diagnosis of dementia recommends neuroimaging at the time of initial dementia assessment "under most circumstances" [20]. Earlier published guidelines state that neuroimaging is optional [29], and some offer prediction rules that dictate when imaging is indicated [19,30]. A recent evaluation of six published sets of prediction rules for neuroimaging in the evaluation of dementia found that the sensitivity of these rules for identifying potentially reversible causes of dementia, such as subdural hematomas, neoplasm, or hydrocephalus, ranged from 12.5% to 100%. Depending on the rules used, it was estimated that 12% to 88% of patients with a potentially reversible cause of dementia would not be imaged [31]. Another study evaluating the usefulness of the prediction rules from the American Academy of Neurology guidelines published in 1994 [30] found that 5% of cases with a meaningful lesion found on neuroimaging had none of the clinical predictors [32]. Although the guidelines support the use of either a non-contrast-enhanced CT scan or MRI, MRI is more sensitive than CT for identifying small cerebrovascular lesions and space-occupying lesions that could cause cognitive impairment. The clinical significance of multiple small white matter changes seen on T2-weighted MRI images in the elderly is often uncertain, however.

Neuroimaging is essential for the diagnosis of cerebrovascular dementia. Clinically silent (ie, no recognized focal event) lacunar infarcts, ischemic white matter changes, and even cortical infarctions that affect cognition may be present. Imaging analysis techniques that quantify the volume of brain structures or lesions may be useful in the future for diagnosing AD [33].

Presently, these techniques are expensive and time-consuming. Neither single photon emission CT nor positron emission tomography is recommended for routine use in the diagnostic evaluation of dementia [20]. These studies can be useful diagnostic adjuncts, however.

Laboratory tests

Laboratory tests should be performed to identify infectious, metabolic, toxic, and inflammatory disorders that can cause neuropsychologic impairment. Required tests include a complete blood cell count; tests of electrolyte, serum glucose, vitamin B_{12}, and folate levels; tests of liver, renal, and thyroid function; and a serologic test for syphilis [19,20]. In a recent study, the use of these tests affected patient management in 13% of consecutive patients being evaluated for dementia [32].

Further diagnostic testing should be based on clinical suspicion. This includes serum or urine tests for toxins, drugs, or heavy metals when exposure is suspected; sedimentation rate for suspected infectious or inflammatory disorders; and human immunodeficiency virus antibody testing. Genetic testing may be useful in those with three or more first-degree relatives with a dementing illness. Assuming that mass lesions are absent, a lumbar puncture may help to diagnose metastatic cancer, infection, vasculitis, encephalitis, meningitis, syphilis, or hydrocephalus. Lumbar puncture may be particularly useful in dementia patients less than 55 years of age or in those with rapid progression, unusual dementia, or immunosuppression [30]. Specific tests on cerebrospinal fluid (CSF), although not standard, may be helpful for diagnosis. For example, elevation of the normal brain protein 14-3-3 in the CSF of patients with progressive dementia without CSF pleocytosis has been reported to be 96% sensitive and 99% specific for Creutzfeldt-Jakob disease [20,34]. An electroencephalogram may identify the periodic sharp wave complexes associated with Creutzfeldt-Jakob disease and may be helpful in distinguishing depression or delirium from dementia [30].

Differential diagnosis of memory and cognitive impairment

Not all patients with complaints of memory loss have dementia. Some have no memory loss at all, whereas others have a mild degree of impairment insufficient for a diagnosis of dementia. When evaluating the patient with complaints of memory loss, the physician must first determine whether true memory loss is present. A number of conditions can lead to memory complaints or cognitive impairment. These are listed in Table 2.

Cognitive changes with normal aging

Cognitive decline related solely to aging remains a controversial topic. Normative data from cross-sectional studies examining neuropsychologic

Table 2
Problems presenting as memory loss

Dementia
Worried well
Normal aging
Depression
Delirium
Stroke syndromes
Bradykinesia
Abulia
Seizure
Excessive daytime somnolence
Amnestic syndrome

performance demonstrate declines in memory with age. Specifically, it has been reported that new learning ability or acquisition declines with age, whereas cued recall remains stable [35]. The pattern and severity of deficits across cognitive domains vary widely depending on the population studied, the tests used, and whether the data are cross-sectional or longitudinal [35–37]. A clear conclusion from studies of neuropsychologic function in the elderly is that aging-related declines are not inevitable, and that when they do occur, it is age-related diseases that are often responsible [36,38]. It should be emphasized that all patients with memory complaints need a careful evaluation. It is not appropriate to assume that memory complaints, at any age, are caused by senescence.

Mild cognitive impairment

One of the more important clinical concepts to emerge recently in the field of cognitive disorders is MCI. Although exact definitions vary, MCI exists in patients with memory complaints and objective memory impairment. Because the activities of daily living are intact in these patients, they do not meet the criteria for dementia. This condition is considered to be a transitional stage between normal aging and dementia. The significance of MCI lies in the identification of patients at high risk for developing dementia and in the potential for treating these patients so as to prevent further decline. Guidelines for the detection and management of this condition have been published recently [39]. It is reported in this evidence-based review that subjects with MCI followed for up to 4 years have a high risk of progressing to dementia, with an annual conversion rate ranging from 6% to 25%. The recommendations are made that persons with MCI be recognized and monitored for cognitive and functional decline because of their increased risk of progressing to dementia and that general cognitive screening instruments be considered for identifying dementia in patients with MCI [39]. Arguments countering these recommendations question the benefit of clinical monitoring considering the fact that not all those with MCI evolve to dementia and

suggest that the stigma attached to this label could actually work to the patient's detriment [40].

Depression

Memory impairment is commonly associated with major depression in the elderly and may be the presenting symptom of this common and treatable disorder [41]. In fact, memory complaints in the elderly may be more related to depression than to objective memory impairment [42]. The essential features of major depression are sadness that is persistent or anhedonia (loss of interest in usual activities) [5]. In addition to memory impairment and poor concentration, depressed patients experience sleep and appetite disturbances, loss of energy, psychomotor retardation, feelings of worthlessness or guilt, or recurrent thoughts of death. Clinical screening tools for depression are available, such as the Hamilton Scale or the Geriatric Depression Scale [43,44].

Patients with depression generally have impaired recall with relative sparing of recognition memory. The cognitive and mood symptoms can resolve completely with treatment, although response to treatment may be difficult to assess when dementia and depression coexist. Additionally, because depression with cognitive impairment may presage the development of dementia, it is important to follow patients so as to assess treatment effectiveness and track progression of cognitive deficits [45].

Delirium

Delirium or acute confusional state is another condition that is common in the elderly. According to the DSM-IV [5], delirium requires the following:

1. Disturbance of consciousness (ie, reduced clarity of awareness of the environment) with reduced ability to focus, sustain, or shift attention.
2. A change in cognition (eg, memory deficit, disorientation, language disturbance) or the development of a perceptual disturbance that is not better accounted for by a preexisting, established, or evolving dementia.
3. The disturbance develops over a short period (usually hours to days) and tends to fluctuate during the course of the day.

Illusions and hallucinations may be seen, speech may be incoherent at times, sleep-wake cycles are often disturbed, and psychomotor activity is increased or decreased. Delirium is associated with a variety of systemic illnesses, infections, and toxic and metabolic disturbances. Hospitalized patients in general and surgical patients in particular are prone to delirium. Features that help to differentiate delirium from dementia include rapid onset, short duration, and disturbance of consciousness that often waxes and wanes between agitation and lethargy. It is important to recognize that patients with dementia are at increased risk for delirium and that delirium and dementia may coexist [46].

Amnestic syndromes

Amnesia is defined as an inability to learn new information and is an early sign of AD. Unlike AD, however, the amnestic syndromes are characterized by isolated amnesia with preservation of other areas of neuropsychologic function, such as language and visuospatial ability. Amnestic syndromes are generally associated with conditions that affect the mesial temporal lobes and their connections with the fornix, mamillary bodies, and thalamus. Causes of amnesia include Korsakov's syndrome associated with alcohol abuse and thiamine deficiency, herpes encephalitis, and head trauma [47].

It is important to recognize that patients with isolated memory loss without apparent cause are at high risk for developing dementia and should be closely monitored [48].

Aphasia

Language disturbance or aphasia complicates the evaluation of the cognitively impaired patient. An aphasic patient may be unable to participate in memory testing because of an inability to comprehend instructions, repeat words or phrases, or read. Aphasia is associated with dysfunction in the dominant hemisphere, usually the left hemisphere. Anterior lesions cause nonfluent aphasia with sparse verbal output, whereas posterior lesions in Wernicke's area are associated with fluent verbal output with word substitutions or paraphasias and impaired comprehension [49]. Dominant hemisphere strokes are the most common cause of aphasia; however, anomia may be the earliest feature of AD or frontal lobe degenerative dementia [21].

Etiology of dementia

Once it has been determined that a patient's complaints of memory loss are the result of dementia, the physician must determine the nature of the dementing disorder. Table 3 provides a partial list of the many causes of dementia. The following discussion highlights some of the more common and reversible causes. Detailed information on some of these conditions may be found in other articles in this issue. Comprehensive reviews of the causes of dementia are also available [4,18].

Identifying less common causes of dementia

Although AD is the most common cause of dementia, the physician must be vigilant to less common causes. Clues to diagnosing less common conditions include the presence of focal neurologic findings that suggest focal structural lesions, such as stroke, tumor, or subdural hematoma. The early onset of behavioral abnormalities, language dysfunction, rigidity, or apraxia suggests frontotemporal dementia. Parkinsonian features in early stages of the disease suggest the presence of Parkinson's disease or dementia with

Table 3
Causes of dementia

Cortical degenerative dementias	Dementias associated with parkinsonism
Alzheimer's disease	Parkinson's disease
Frontotemporal dementia	Dementia with Lewy bodies
Vascular dementias	Progressive supranuclear palsy
Multiple large vessel infarcts	Multiple systems atrophy
Single strategic infarct	Cortical-basal ganglionic degeneration
Lacunar state	Idiopathic basal ganglia calcifications
Binswanger's disease	Parkinsonism-dementia complex of Guam
CADASIL[a]	Other extrapyramidal disorders
Toxic/metabolic conditions	Wilson's disease
Medication-induced dementia	Huntington's disease
Alcohol-related dementia	Hallervorden-Spatz disease
Dementia related to heavy metal exposure	Dementias related to infections
	Prion diseases
Vitamin B_{12} deficiency	Creutzfeldt-Jakob Disease
Folate deficiency	Gerstmann-Straussler-Scheinker Disease
Hypo- or hyperthyroidism	Kuru
Hypo- or hyperparathyroidism	New-variant Creutzfeldt-Jakob Disease
Hypo or hypermagnesemia	Neurosyphilis
Hypo or hypercalcemia	AIDS dementia
Cushing's disease	Chronic meningitis
Addison's disease	Fungal
Renal failure	Tuberculosis
Liver failure	Lyme disease
Porphyria	Viral encephalitis
Domoic acid poisoning	Whipple's disease
Paraneoplastic syndromes	Trauma-related dementias
Limbic encephalitis	Dementia related to closed-head injury
Autoimmune/inflammatory disorders	Chronic subdural hematoma
Multiple sclerosis	Dementia pugilistica
Behcet's disease	Miscellaneous disorders
Lupus erythematosus	Normal pressure hydrocephalus
Sarcoidosis	Hippocampal sclerosis
Temporal arteritis and other central nervous system vasculitides	Central nervous system tumors
	Mitochondrial encephalopathies

[a] Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy.

Lewy bodies. Fluctuations in performance, often over a period of minutes, or the presence of visual hallucinations also suggests dementia with Lewy bodies. Age of onset less than 50 years or the presence of three or more first-degree relatives with dementia suggests a genetic dementing disorder.

Alzheimer's disease

AD is the most common cause of dementia, comprising over two thirds of all cases in most studies [50]. There is no confirmed biologic marker for AD; however, standardized clinical criteria have improved the accuracy of diagnosis to greater than 85% [8,51]. Onset is insidious, and progression

slow. Language disturbances may be an early feature, with anomia progressing to fluent aphasia with word substitutions or paraphasias and impairment in comprehension. Recent memory and the ability to learn new information are also impaired early in AD. Other common disturbances in cortical function include apraxia (impaired ability to perform motor activities despite intact motor function), agnosia (inability to recognize objects despite intact sensory functioning), and alexia (inability to comprehend the written word). Patients with AD commonly lack knowledge of their memory problems. Delusions and hallucinations are common and occur in up to 50% of patients [52]. The results of the elementary neurologic examination are remarkably normal in most cases. Subtypes of AD include those with isolated cognitive impairments that later progress to more widespread cognitive deficits. In addition, another type presents with rigid or bradykinetic symptoms resembling Parkinson's disease. When present early, extrapyramidal signs suggest a more rapid decline [53]. In those patients with three or more first-degree affected family members, familial AD must be suspected, and appropriate testing should be undertaken.

Vascular dementia

The concept of (VsD) is evolving as it becomes increasingly recognized that in addition to causing cognitive decline alone, vascular lesions modify the expression of Alzheimer pathologic findings [54]. VsD is clinically recognized in patients with a prior history of strokes, focal neurologic findings, or strokes on neuroimaging. The classic sudden onset with stepwise deterioration of VsD probably occurs in less than half of cases. Because of these clinically silent cases, it is important to obtain neuroimaging in all cases of dementia. The cognitive deficits associated with VsD depend on the lesion location. Large vessel strokes cause cortical deficits, such as aphasia and focal neurologic deficits, such as hemianopia and hemiparesis. Multiple small vessel strokes cause a subcortical clinical presentation with forgetfulness and prominent executive function deficits. Pseudobulbar palsy, gait disturbances, and urinary incontinence are also common. Although several sets of criteria exist for VsD [5,9,55], the clinician should be concerned with stroke prevention in any dementia patient with cerebrovascular risk factors or vascular lesions on neuroimaging.

Frontotemporal dementia

Frontotemporal dementia consists of a clinically and pathologically heterogeneous group of disorders, including Pick's disease. These have in common degeneration of the frontal and temporal lobes. Diagnostic criteria for frontotemporal dementia are listed in Table 4 [56]. Behavioral changes, including disinhibition, impulsiveness, social inappropriateness, apathy, and withdrawal, are early and prominent features. These behavioral changes provide the most important clue allowing the differentiation of this condition

Table 4
Criteria for the diagnosis of frontotemporal dementia

A. Dominant deficits in behavior and conduct appearing early in the course
Loss of personal awareness (neglect of hygiene and grooming)
Loss of social graces and awareness
Disinhibition (sexually provocative or demanding, inappropriate jocularity), overactivity, or restlessness, often with a stereotyped repertoire
Impulsivity, distractibility
Hyperorality (dietary changes, excessive eating, smoking, or alcohol consumption, preference for sweet foods or food fads, oral exploration of objects)
Withdrawal from social contact, apathy or inertia
Stereotyped or perseverative behaviors (wandering, repetitive clapping, humming or singing, ritualistic toileting, dressing)
B. Speech output changes
Progressive reduction of speech (late mutism, economy of speech)
Stereotypy of speech (few repeated phrases or themes), perseveration
Echolalia
C. Physical signs
Early or prominent primitive or "frontal" reflexes
Early incontinence
Late akinesia, rigidity, tremor
D. Deficits in social comportment, behavior, judgment, or language are out of proportion to memory deficit. Memory loss is variable, often seems to result from lack of concern or effort; frontal lobe impairments most notable: abstraction, planning, self-regulation of behavior

from AD. Language disturbances may also appear early, whereas visuospatial function remains intact until later in the illness [57]. Neuroimaging may allow the visualization of focal atrophy, but the disease can often be recognized clinically before changes on routine imaging are apparent [58]. Single photon emission CT imaging demonstrates hypoperfusion in the frontal and temporal lobes before atrophy in these regions is evident on structural imaging [58].

Dementias associated with parkinsonism

Parkinsonism is a syndrome of disturbed motor function characterized by bradykinesia, muscle rigidity, rest tremor, and postural instability. Dementia is often a secondary feature. There are many diseases that cause parkinsonism. The most common of these is Parkinson's disease, which is characterized by loss of dopaminergic neurons in the substantia nigra of the midbrain. The presence of Lewy bodies, target-shaped inclusions with a dense eosinophilic core, within the degenerating neurons of the substantia nigra confirms the diagnosis of Parkinson's disease; however, these lesions may also occur in the cortex. Dementias associated with Lewy bodies comprise a spectrum of diseases, including Parkinson's disease with Lewy body pathologic changes limited to the brain stem, dementia with Lewy bodies associated with Lewy bodies in the cerebral cortex, and AD associated with cortical Lewy bodies as well as typical Alzheimer pathologic findings. Dividing lines between these conditions are somewhat arbitrary;

however, the practitioner should be familiar with the basic clinical distinctions, because the correct diagnosis affects patient management.

Dementia occurs at some time during the course of the illness in approximately 40% of patients with Parkinson's disease [59]. Cognitive decline begins at least 1 year after the onset of the movement disorder and is associated with impaired recall that is aided with recognition cues, prominent executive function deficits, and intact language [60]. Dementia with Lewy bodies, in contrast to Parkinson's disease, is identifiable by fluctuating cognitive performance, well-formed visual hallucinations unrelated to dopaminergic therapy, and parkinsonism that emerges simultaneously with the cognitive impairment. These features allow it to be differentiated from AD and Parkinson's disease. It is particularly important to recognize dementia with Lewy bodies because of the severe adverse reactions that these patients may have to neuroleptic medications used to treat behavioral problems. Because of this, neuroleptics should be avoided in the treatment of this disease [61]. Diagnostic criteria for identifying dementia with Lewy bodies are listed in Table 5 [62]. Other degenerative parkinsonian syndromes are much less common. Progressive supranuclear palsy begins at the age of 40 years or older and is characterized by a rigid-akinetic form of parkinsonism, dementia, supranuclear gaze palsy, severe dysarthria, neck rigidity (usually in extension), minimal tremor, and frequent falls [63]. Multisystem atrophy refers to a group of adult-onset progressive neurodegenerative disorders (ie, striatonigral degeneration, Shy-Drager syndrome, olivopontocerebellar atrophy) characterized by parkinsonism that

Table 5
Criteria for diagnosing Lewy body dementia

A.	The central requirement is progressive cognitive decline of sufficient magnitude to interfere with normal social or occupational function. Prominent or persistent memory impairment may not necessarily occur in the early stages but is usually evident with progression. Deficits on tests of attention and frontal-subcortical skills and visuospatial ability may be especially prominent.
B.	Two of the following are required for a probable diagnosis, and one for a possible diagnosis of dementia with Lewy bodies:
	Fluctuating cognition with pronounced variations in attention and alertness
	Recurrent visual hallucinations that are typically well formed and detailed
	Spontaneous motor features of parkinsonism
D.	Features supportive of the diagnosis are as follows:
	Repeated falls
	Syncope
	Transient loss of consciousness
	Neuroleptic sensitivity (deterioration in cognitive function, parkinsonism, drowsiness, and some features of so-called neuroleptic malignant syndrome)
	Systematized delusions
	Hallucinations in other modalities
E.	A diagnosis of dementia with Lewy bodies is less likely in the presence of the following:
	Stroke disease, evident as focal neurologic signs or on brain imaging
	Evidence on physical examination and investigation of any physical illness or other brain disorder sufficient to account for the clinical picture

is poorly responsive to levodopa and is associated with cerebellar dysfunction, pyramidal dysfunction, or symptomatic autonomic failure as well as dementia [64]. Vascular parkinsonism is characterized by the stepwise progression of an akinetic-rigid syndrome in the setting of clinical strokes or other vascular risk factors, such as hypertension, diabetes, or lipid abnormalities. Clinical signs may improve without the use of levodopa [65]. Cortical-basal ganglionic degeneration is characterized by a chronic progressive akinetic-rigid parkinsonian syndrome resistant to levodopa and is associated with dystonic limb posturing and focal myoclonus. There is also evidence of higher cortical dysfunction (apraxia, cortical sensory loss, or alien limb) [66].

Dementia related to chronic subdural hematoma

The presenting features of chronic subdural hematoma include focal symptoms, headache, or cognitive/personality changes. Without neuroimaging, the diagnosis is rarely straightforward. A history of trauma is absent in one third of patients. The onset may be sudden with a fluctuating course or may extend over weeks to months. Seventy percent of chronic subdural hematomas occur in patients more than 60 years old, and men are more commonly affected than women [67–69]. Patients may have lethargy or agitation. Cognitive deficits involve multiple domains, including recent memory, language, abstract thinking, calculations, and judgment [68,69]. A contrast-enhanced brain CT scan may be required to recognize chronic subdural hematoma, because the density of the lesion may be the same as that of brain parenchyma. Medical management is recommended for small lesions with minimal clinical signs [70]. Surgical management is indicated for the rest. Either burr holes or twist-drill craniotomy is effective and associated with relatively few complications [71].

Dementias associated with infectious disease

The prion diseases, including Creutzfeldt-Jakob disease and dementia secondary to human immunodeficiency virus infection, are covered elsewhere in this issue. Creutzfeldt-Jakob disease usually presents with a dementia that progresses over weeks or months. In addition to cognitive impairment, many patients experience depression or emotional lability. Myoclonus, especially in response to stimuli, is seen later in the course of the disease in three fourths of patients. Ataxia is often seen as a late manifestation of the disease. Gait and vision may be affected. The dementia is often rapidly progressive, with the median time from onset of symptoms to death being 4.5 months. Diagnosis may be aided by the electroencephalographic finding of 1- to 2-Hz triphasic sharp waves that are often asymmetric. Elevated levels of the 14-3-3 protein in CSF in the absence of pleocytosis also support the diagnosis. Recently, variant Creutzfeldt-Jakob disease has been found after consumption of beef infected by bovine spongiform encephalopathy. These variant cases generally affect younger patients and have a more prolonged course.

General paresis refers to the dementia associated with parenchymatous neurosyphilis. Once a common cause of institutionalization, general paresis is now rarely seen, causing some to question the utility of routinely screening for treponemal infection [20]. Onset may occur many years after the initial infection. Patients exhibit memory impairment with confabulation, dysarthria, impaired judgment, psychosis, and grandiosity [4]. Treponemal serology tests, such as the micro hemagglutination assay for treponema pallidum (MHA-TP), have fewer false-negative results than nontreponemal tests, such as the venereal disease research laboratory slide test (VDRL). Cases screening positive by serologic testing should undergo lumbar puncture. The CSF VDRL test is highly specific; however, the results may be negative in some cases. Patients with positive serology and elevated white blood cells in their CSF should be treated. Aqueous penicillin G remains the treatment of choice.

Toxin-related dementias

Wernicke's encephalopathy, characterized by delirium, ophthalmoplegia, and ataxia, is related to thiamine deficiency and is associated with prolonged heavy use of alcohol. Prompt thiamine replacement may reverse the delirium and other signs, although some patients still evolve to Korsakoff's syndrome, an amnestic disorder that may be permanent [72]. Although the DSM-IV recognizes alcohol-induced dementia that persists after heavy drinking, the lack of pathologic findings in the brain related to alcohol abuse has called into question the direct toxic effect of alcohol on the brain [5,73]. The increased risk of head injury and association of heavy prolonged alcohol use with blood disorders that can lead to stroke may also cause persistent cognitive impairment.

Medications are a common cause of delirium and cognitive decline in the elderly and may be responsible for 1.5% to 10% of all clinically diagnosed dementias [74]. Patients with dementia may be particularly susceptible to further cognitive impairment with medication use [75–77]. Sedatives and hypnotics (eg, benzodiazepines), anticholinergic drugs (eg, trihexyphenidyl and meclizine), antidepressants (eg, amitriptyline), analgesics, and antihypertensives (eg, beta-blockers) are all commonly prescribed medications that can cause cognitive impairment reversible on withdrawal of the medication [18]. Medications used to treat the behavioral complications of dementia, such as the antipsychotics haloperidol and thioridazine, may worsen the memory impairment. The lowest possible dose should be used to control the target symptoms, and the need for continued use of the medication should be assessed at regular intervals.

Dementia associated with metabolic disturbance

Neurologic symptoms related to vitamin B_{12} deficiency occur most commonly in the fourth through sixth decades and include paresthesias of the

feet and hands, loss of vibratory and position sensation, ataxia, extremity weakness, and neuropsychiatric disturbances. Confusion, memory loss, paranoia, hallucinations, depression, and irritability are all features of the neuropsychiatric syndrome that has been described as megaloblastic madness [78,79]. Importantly, among those with cognitive and behavioral symptoms and vitamin B_{12} deficiency, as many as one fourth do not have the megaloblastic anemia that is classically associated with pernicious anemia [80]. Focal areas of cerebral demyelination characterize the brain pathologic findings much like the loss of myelin in the posterior and lateral columns of the spinal cord associated with this syndrome. Treatment consists of intramuscular administration of vitamin B_{12}. This usually results in improvement in the motor and sensory deficits and has been reported to improve language and frontal function in individuals with vitamin B_{12}–related cognitive impairment [81]. Folate deficiency may cause a similar syndrome that can be treated with oral folic acid supplements.

Dementia associated with hypothyroidism is characterized by inattention, memory impairment, and impaired abstraction. Psychosis may occur [4]. Owing partly to the common assessment of thyroid function, the myxedema syndrome of edema, weight gain, thick skin, cold intolerance, constipation, and psychomotor slowing is rarely seen. More often, clinicians encounter patients with elevated thyrotropin levels, normal thyroid hormone levels, and absent or mild symptoms [82]. Evidence from community-based studies demonstrates an association between this condition known as subclinical hypothyroidism and cognitive impairment [83]. Furthermore, treatment with thyroxine has been associated with significant improvement in cognitive function [82].

Normal pressure hydrocephalus

Normal pressure hydrocephalus is a rare condition characterized by the triad of dementia, gait disturbance, and urinary incontinence. Onset is rare before 60 years of age. Typically, the gait disturbance develops first, followed by dementia. Incontinence may not occur until the later stages of the disease. The gait is associated with slow and small steps akin to parkinsonism, with slow initiation giving the appearance that the feet are stuck to the floor–hence, the term *magnetic gait*. The dementia syndrome is mild, with forgetfulness, psychomotor slowing, and impaired executive function being the predominant symptoms. The cause is unknown but is thought to be related to ischemic demyelination in periventricular white matter secondary to a combination of vascular insufficiency and intermittent slight increases in CSF pressure [84]. The diagnosis is made by neuroimaging in patients with the appropriate clinical picture. MRI or CT demonstrates ventricular dilatation, especially of the frontal horns, that is out of proportion to the amount of cortical sulcal widening. Radionuclide cisternography, once the diagnostic test of choice, is an unreliable predictor of treatment response and is unlikely to contribute to the

diagnostic certainty beyond a good history, examination, and structural brain imaging. If hydrocephalus is a serious consideration, neurologic or neurosurgical consultation is recommended for several invasive tests that do have prognostic value, such as serial lumbar punctures, continuous CSF drainage, and continuous intracranial pressure monitoring. Approximately 30% to 40% of patients have improvement in cognitive function after shunt surgery [85,86]. The elderly, however, are susceptible to complications of shunt surgery, which occur in 30% to 40% of patients, with 6% to 8% having serious complications such as death or residual neurologic deficits. Predictors of good response to shunting include a short history of mental decline, known cause of hydrocephalus (eg, subarachnoid hemorrhage or meningitis), predominant gait disorder, and clinical improvement after serial lumbar CSF taps or continuous drainage [86].

Summary

The initial approach to the patient with memory complaints should consist of a focused history, mental status examination, and functional assessment. Patients with MCI should be monitored every 6 to 12 months for conversion to dementia. Delirium, depression, amnestic disorders, and aphasias should be considered in the differential diagnosis of memory impairment. Once a diagnosis of dementia is made, patients should have a brain CT or MRI scan and laboratory tests to assist with determining the cause.

It is crucial that dementia be recognized and evaluated at the earliest stage so as to begin appropriate therapy and allow the patient to have a role in management decisions. In the future, therapies for MCI may prevent conversion to dementia. The need for early recognition makes the development of diagnostic tools, such as quantitative or functional neuroimaging, and genetic or clinical biologic markers essential.

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Relationship Between Caffeine Intake and Parkinson Disease

To the Editor: The article by Dr Ross and colleagues[1] was widely reported to show that coffee is an independent "protective factor" against the development of Parkinson disease (PD). Using a prospective longitudinal epidemiological database, the authors did, in fact, find an inverse association between higher caffeine intake and risk of developing PD. However, association does not prove causation. They did not consider another potential explanation of this association; namely, that incipient or preclinical PD causes decreased novelty-seeking behaviors.[2,3] Several studies have shown inverse associations between tobacco, alcohol, and coffee intake and PD,[1-3] and it is more plausible that these various substances (including other caffeinated beverages such as tea, cola, and chocolate) are underused by persons with incipient PD, rather than that each agent independently protects against PD.

Patients destined to develop clinical PD may have a presymptomatic period of 5 to 20 years, during which they may manifest early changes of nigrostriatal dopaminergic deficiency characterized by decreased motor facility and speed and decreased cognitive speed and initiative.[4,5] Thus, it is unsurprising that shorter intervals between assessment of "risk-factors" and clinical diagnosis (eg, 10.6 vs 16.6 years[1]) are associated with a greater inverse association with PD. Such an effect could also possibly reflect increased "protective" effect of a proportionally small increment in total coffee-drinking years (eg, 43 to 60 years vs 37 to 54 years assuming onset of coffee-drinking at age 16 years). However, Ross et al did not find a dose-response relationship between coffee intake and risk of PD. The adjusted relative hazards in their Tables 1 and 2 show the largest proportional changes in risk between individuals who drink caffeinated beverages and those who do not, with little change in risk ratios for intakes between 4 and 24 oz/d.

While the authors speculate that incipient PD causes "physiological intolerance" to caffeine, individuals with preclinical PD likely have a neurophysiologically based disinclination to use of caffeine, alcohol, and tobacco. Thus, numerous studies find inverse associations between intake of these agents and later diagnosis of PD, although there is no clinical evidence that caffeine or nicotine deter or contribute to PD. (Indeed, limited clinical trials of nicotine have shown no beneficial effects.) It is much more likely that PD "protects" against these discretionary habits.

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1. Ross GW, Abbott RD, Petrovitch H, et al. Association of coffee and caffeine intake with the risk of Parkinson disease. *JAMA*. 2000;283:2674-2679.
2. Honig LS. Smoking and Parkinson's disease. *Neurology*. 1999;53:1158.
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In Reply: We agree with Dr Honig that "association does not prove causation," and we clearly stated that the observational nature of our study does not support the conclusion that coffee or caffeine are protective against PD. How others report or interpret our results is beyond our control.

We also agree that very early PD may affect the development of habits such as coffee drinking, cigarette smoking, and alcohol consumption that characteristically begin in late adolescence or early adulthood. As Honig points out, we noted that individuals with a constitutional propensity to develop PD could have a physiological intolerance to caffeine. Honig states that individuals with preclinical PD may have a "neurophysiologically based disinclination" to caffeine use. The distinction between "physiological intolerance" and "neurophysiologically based disinclination" is not clear to us. We also disagree with Honig's statement that patients destined to develop PD are symptomatic for 5 to 20 years preceding diagnosis, as there is no evidence for this assertion.

Dose-response relationships for coffee and caffeine are clearly shown in Tables 1 and 2 of our article and are evident throughout the entire range of coffee intake. The low numbers that Honig refers to in the highest consumption category ($\geq$ 28 oz/d) represent a simple continuation of the trend of lower PD risk observed in men who consumed greater amounts of coffee. The number of subjects at risk in the highest consumption category is not low, and we fail to follow Honig's logic for excluding these. Even if we exclude the men who drank the most coffee, a significant dose response remains with or without adjustment for age and pack-years of smoking ($P<.01$).

Although an association between coffee drinking and PD has been noted in some case-control studies, no other longitudinal studies (including the Rotterdam study[1] referenced by Honig) have found a statistically significant relationship. We agree that

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Letters Section Editors: Stephen J. Lurie, MD, PhD, Senior Editor; Phil B. Fontanarosa, MD, Executive Deputy Editor.

the observation only raises questions about the possible cause. Whether the association between coffee and PD is the result of early disease-related avoidance of coffee or caffeine, a pharmacologic effect, a true protective effect, or some other mechanism, it remains a potential clue to understanding disease processes and warrants further research.

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1. Willems-Giesbergen PC, de Rijk MC, van Swieten JC, Hofman A, Breteler MM. Smoking, alcohol and coffee consumption and the risk of PD: results from the Rotterdam Study. *Neurology*. 2000;54:A347-A348.

Aspirin in Acute Ischemic Stroke

To the Editor: In their Editorial, Drs Mayberg and Furlan[1] indicate that the current evidence does not allow clinicians to decide whether tissue-type plasminogen activator (tPA) or ancrod is better for patients with acute ischemic stroke. A more basic question is whether either treatment is better than aspirin administered in the same time period. In these trials, patients randomized to the placebo group were prohibited from receiving any antithrombotic treatments for the first 24 hours in the study of tPA[2] or for the first 5 days in the study of ancrod.[3] Do these protocols reflect the standard of care for such patients? Evidence now exists that aspirin may be beneficial in patients with acute ischemic stroke,[4,5] but information about its administration in the first 3 hours after the onset of symptoms is limited. The question of which treatment is best needs to be addressed because a treatment as simple, inexpensive, and safe as aspirin would have broad application.

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1. Mayberg MR, Furlan A. Ancrod—is snake venom an antidote for stroke? *JAMA*. 2000;283:2440-2442.
2. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med*. 1995; 333:1581-1587.
3. Sherman DG, Atkinson RP, Chippendale T, et al. Intravenous ancrod for treatment of acute ischemic stroke: the STAT study: a randomized controlled trial. *JAMA*. 2000;283:2395-2403.
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In Reply: The purpose of our Editorial was to discuss the results of the ancrod trial and not the relative efficacy of aspirin in acute stroke. Aspirin has not been approved by the US Food and Drug Administration to treat acute stroke.

The relative benefits and safety of aspirin either combined with or in comparison to ancrod, tPA, or any other new stroke therapy can only be determined from a randomized controlled trial.

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Screening for Depression in Primary Care

To the Editor: In his Clinical Crossroads discussion of a 52-year-old suicidal man,[1] Dr Jacobs rightly emphasizes the importance of suicide risk assessment by the primary care physician. Unfortunately, he endorses a broad program of screening for depression, noting that "Depression screening is simple, cost-effective, reliable, and potentially money saving. . . ." He notes that the suicidal ideation of the patient presented "would have been detected by a primary care physician using a depression screening tool." However, there are no data to support either statement, nor was there evidence that this suicidal patient had contact with a primary care physician during which an opportunity for suicide risk assessment was missed. Jacobs reaches the erroneous conclusion that the complex problems of detecting and treating depression or suicide can be addressed with simple screening maneuvers, a conclusion for which there is no empiric basis.[2]

Because the natural history of major depressive disorder and its associated suicide risk has a waxing and waning course in patients who present to primary care physicians, screening is more likely to be inaccurate in primary care than psychiatric settings.[3] All screening questionnaires have high sensitivity and poor specificity when used in primary care settings, thus leading to poor positive predictive value. Essentially, all questionnaires measure daily stresses and mood, rather than stable depressive symptoms. The criterion-based diagnoses detected are often mild and cause minimal impairment, thus leading to uncertainties regarding the appropriate course of treatment.[4]

Patients in primary care typically have a high level of resistance to routine queries about depressive symptoms, as compared to the more receptive attitude of the occasional patient who is truly suicidal. In short, the depressed patient seen in primary care is far different from the one seen in psychiatry, and the appropriate approach to diagnosis and treatment is unclear.[5] The same uncertainties apply even more to the detection of active suicidal ideation, which is rarely seen in routine primary care practice and for which effective detection and prevention programs have not been demonstrated.

The foregoing should not be a reason for negativity, which is neither compassionate nor productive, but rather skepticism regarding recommendations for broad screening programs. There is no evidence that such programs save either money or lives, de-